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THE EFFECT OF TEMPERATURE ON THE RATE OF SETTING OF PLASTER OF PARIS[1]

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INTRODUCTION

Most users of plaster of Paris have observed that the setting rate can be increased by using warm water for mixing or in some way heating the mass during setting. So far as the writer is aware, however, no systematic study has been made of the effect of temperature on the time of setting. This paper records the results of observations made on 6 dental plasters over a temperature range of 10 to 100°C. (50 to 212°F.). The plasters were chosen as representing typical fast and slow setting imported and local products.

EXPERIMENTAL TECHNIQUE

Small mixes were used, with water and plaster in the ratio 1:2 by weight. Standard Gillmore needles were employed to indicate the progress of setting. In the early experiments the plaster and water were placed in a box which was heated to the test temperature and, after allowing ample time for the temperature to be stabilized, the plaster and water were spatulated together in that particular atmosphere. This seemed to give satisfactory results for temperatures up to about 50°C. (122°F.) but, at higher temperatures, it was obvious that evaporation from the surface of the mixed plaster mass affected the results considerably. There was evidence that the slower setting samples hardened as much by loss of water as by the actual setting reaction. Attempts were made to overcome this evaporation effect by carrying out the experiments in a steam atmosphere in a heated water bath. The plaster was mixed in a rubber cup (both the water and plaster having been heated previously just slightly above the temperature of the test) and the container and plaster mix were left in the water bath surrounded by the steam generated from the hot water. These conditions did seem to reduce the amount of water lost by evaporation, but they did not overcome the tendency, in the tests made at the higher temperatures, for the surface layers of the plaster mix to cool slightly while the Gillmore test was being made. This resulted in the surface layers hardening at a slightly greater rate than the underlying plaster.

It was decided finally to carry out the setting time measurements for all temperatures below 100°C. in a bath of liquid paraffin. At temperatures up to 90°C.

[1] Received for publication June 17, 1944.

[2] Research Fellow of the National Health and Medical Research Council of Australia. Proof of this paper was read by Dr. E. W. Skinner, Northwestern University, Chicago.

there seemed to be no reaction between the paraffin and the plaster mix. With the slow setting plasters there was probably a slight interference with the setting of a very thin layer (of the order of 0.02 inch) of the plaster in direct contact with the paraffin. Care was taken to account for this non-hardening "skin" in interpreting the results of the Gillmore tests. The tests at 100°C. were carried out in stoppered weighing bottles immersed in boiling water.

The technique of mixing and test was as follows: The weighed amount of plaster was placed in a stout metal bowl, and quickly heated in an air oven to a temperature very slightly above that at which the setting time was to be determined. The mixing water having been heated to a similar temperature was then added to

TABLE I

Gillmore setting times (in minutes)

(All plasters mixed with water in the ratio 2:1 by weight)

TEMPERATURE		IMPORTED PLASTERS						AUSTRALIAN PLASTERS					
		A		B		C		D		E		F	
°C.	°F.	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
10	50	7	14	10½	19	30	48	—	—	25	40	30	48
15	59	4	8¾	7¼	14	24	42	—	—	—	—	—	—
20	68	2¾	6	5¾	11	20	37	9	17	17	28	22	34
30	86	2½	5	5	10	16½	30	7	13½	15	24	18	28
40	104	2¼	4¾	4¾	10	16	28	6¼	12	15	23	18	28
50	122	2½	5	5	10	16	28	6	12	16	25	19	30
60	140	2¾	6	5¼	10¼	17	31	6	12½	24	40	25	43
70	158	3¾	9	6½	13	29	53	6½	13	63	100*	48	80*
75	167	—	—	7½	17	—	—	—	—	—	—	90*	—
80	176	6½	25	10	27	62	140*	10	21	360*	—	330*	—
85	185	—	—	30*	90*	100*	—	—	—	—	—	—	—
90	194	150*	—	300*	—	>420	—	—	—	NS?	—	NS?	—
100	212	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.

* Signifies approximate value.

> Signifies greater than.

NS. Signifies no setting evidenced.

the plaster, and spatulation carried out as quickly as possible in an atmosphere with a temperature approximating that of the test. It was found convenient to use a stout-walled glass thermometer as the mixing "spatula"; this enabled the operator to follow the slight decrease in temperature which occurred during the fifteen to twenty seconds spatulation. By observing the magnitude of this temperature drop in a preliminary experiment, adjustment could be made for it in the subsequent experiments at any particular temperature. After spatulation, the bowl was tapped to release air bubbles from the plaster mix and to produce a flat surface thereon. The bowl and its contents were immediately lowered into a paraffin bath fitted with a stirrer and facilities for controlling the temperature. (It has been found possible to keep the temperature of the test bath within plus or

minus 1°C. over very long periods). In some experiments a thermometer was immersed in the middle of the plaster mix so that the temperature of the setting plaster could be followed independent of the surrounding paraffin. When the temperature of the plaster was observed to rise above that of the paraffin bath, it was taken as a sure indication that the setting reaction was proceeding.

RESULTS

The mean results of 2 or 3 tests at each temperature are presented in tabular form in Table I. Typical results for a fast and a slow setting plaster are given in graphical form in *fig. 1*. It will be seen that for all 6 plasters there was an appre-

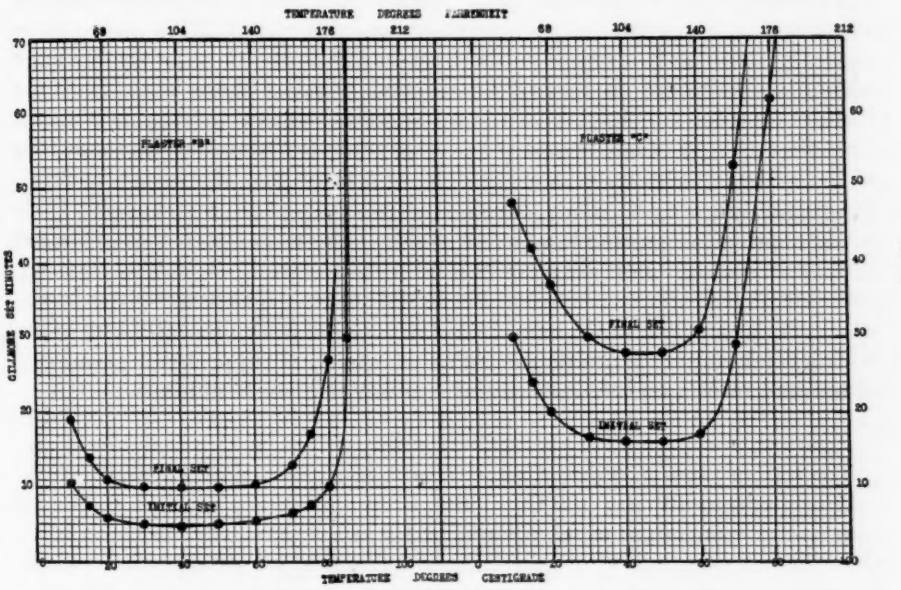

FIG. 1.

FIG. 1

ciable decrease in the setting time (an increase in the setting rate) as the test temperature was increased from 10°C. (50°F.) to about 30°C. (86°F.). Over the temperature range 30 to 50°C. (86 to 122°F.) the setting rates for any particular plaster were practically constant; but, as the temperature was taken above 50 to 60°C. (122 to 140°F.), the setting times began to increase again. With the slow setting plasters the retardation in set became quite marked at 70°C. (158°F.), but temperatures of 80°C. (176°F.) or more were necessary to produce very substantial increases in the setting times of the fast setting materials. In all cases, the setting times increased enormously over the temperature range 80 to 90°C. (176 to 194°F.), and there was some doubt as to whether the slow setting plasters set at all at 90°C.; certainly no setting occurred in the mixes maintained at 100°C.

DISCUSSION

The setting of plaster of Paris, when mixed with water, is generally agreed to be the result of progressive solution of the calcium sulphate subhydrates (mostly hemihydrate) and progressive precipitation and crystal growth of the less soluble dihydrate (gypsum). At least 3 phenomena are involved in this setting reaction:—(a) the solution of the subhydrates; (b) the diffusion of calcium and sulphate ions; and (c) the crystallization of the dihydrate.

The effects which changes in temperature produce on the setting reaction must be largely explainable in terms of changes in the rate of some or all of these phenomena. It seems very likely that the increase in setting rate accompanying the increase in test temperature up to about 40°C. is due to an increased rate of solution of the subhydrates and increased ionic mobility. While these latter probably continue to increase as the temperature is raised, other factors must offset their influence on the setting reaction. One of these is probably the marked decrease in solubility of the subhydrates relative to gypsum. It has been shown (1) (2) that, whereas at average room temperatures the solubilities of the subhydrates in water is of the order of 5 times that of gypsum, at 98°C. the solubilities are the same. Furthermore, at temperatures above 98°C. it would appear that gypsum is no longer stable. Dunn (2) stated that prolonged heating in boiling water will convert it to a subhydrate. It is not surprising, therefore, that the plasters tested at 100°C. did not set. The progressive retardation in the setting rate as the temperature is increased from about 60°C. up to the boiling point of water is probably explainable in terms of the decreasing solubility of the subhydrates and their increasing stability relative to gypsum.

SUMMARY

The setting times of 6 dental plasters have been determined at temperatures ranging between 10° and 100°C. There is an appreciable decrease in setting time as the temperature of the environment is raised from 10°C. to about 30°C. Over the range 30 to 50°C. the setting rate is practically constant and is a maximum for the whole temperature range. At temperatures above 50 to 60°C. the setting times begin to increase again, and with slow setting plasters the retardation becomes quite marked at 70°C. At temperatures above 80°C. all plasters become very slow setting, and at 90°C. there may be little or no evidence of setting at five to six hours. No setting occurs at 100°C. Some explanations of the observed phenomena are offered.

This work was made possible by a grant from the National Health and Medical Research Council of Australia, and was directed by Professor Arthur Amies, whose kindly interest is appreciated. Assistance in the work was afforded by Mr. A. Buchanan, B.Sc., and Messrs. M. Chong and J. Griffith, to whom thanks are due.

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THE NASO-MEATAL LINE AS A GUIDE FOR THE DETERMINATION OF THE OCCLUSAL PLANE[1]

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In determining the level of the occlusal plane in complete denture prosthesis, dentists commonly accept as a guide a line connecting the tragus of the ear (sometimes the auditory meatus) and the ala of the nose, making the occlusal plane of the denture parallel with this line in the lateral aspect, and parallel in the frontal aspect with a line connecting the pupils of the eyes. Reliance upon this naso-meatal line is based upon a considerable number of years of clinical observation; in practice it serves only as a point of departure and is not rigidly binding, i.e., articulation and balance of the artificial teeth ultimately play a larger part in the final determination of the occlusal plane. Wilson (1) and Anthony (2) recommended the guide without comment, while Prothero (3) and Swenson (4) mentioned it but pointed out that it is variable and that other factors enter into the determination of occlusal level. Schlosser (5) suggested a line from the base of the nose to the center of the head of the condyle.

The true usefulness of this guide depends upon two things: whether or not in a representative group of adults having a natural dentition the occlusal plane parallels this line on the average, and whether or not most of the individuals in the group adhere closely enough to the average value to accept that average as a reliable guide.

This study was undertaken to test the usefulness of the clinical rule by determining a quantitative measure of this average and its variability, and to determine what age changes, if any, must be considered.

Fifty-five individuals (22 parents and 33 offspring) were radiographed in the Broadbent-Bolton cephalometer in the Department of Orthodontia, College of Dentistry, University of Illinois, and the lateral head-films so obtained were traced and studied. The convention of treating all points in the lateral head-film as projections to the median sagittal plane was used; when this was done, all bilateral points were treated as single points situated in the midline, and planes became lines lying in the median sagittal plane. The anthropometric plane, proposed by Petrus Camper in 1786, which passes through the center of the external auditory meati and the anterior nasal spine was taken as a bony approximation of the naso-meatal guide line; on the tracings it was necessary to use the superior surface of the ear-rod for the posterior point. The occlusal plane was determined on each tracing by drawing a line through the incisal

[1] Received for publication May 16, 1944.

edge of the maxillary first incisor and through one-half the height of the cusps at the first molars. The angle formed by prolonging these lines was measured with a transparent protractor. Where the lines converged anteriorly, the angle was considered positive; where they diverged anteriorly the angle was called negative. Parents and offspring were treated as two separate groups and the average angular value for each group was determined.

The standard deviation of the distribution was taken to indicate how closely different individuals clustered about the mean value.[2]

The average angle for a group of children having a mean age of 10.1 years was -0.39 ± 0.58 degrees; for a group of adults with a mean age of 40.5 years, 3.91 ± 0.94 degrees. From this it may be seen that the occlusal plane tends more often in early life to parallel the naso-meatal line than it does in adulthood. The difference between the means is of high statistical significance.

The standard deviation for the angle between the naso-meatal plane and the occlusal plane was 4.42, indicating that in a group of adults one might expect 16% to show an anterior divergence of the 2 planes by one-half a degree or more, and another 16% to converge anteriorly by 8.3 degrees or more. Comparison of this standard deviation with those reported by the author (6) for other angles of the craniofacial complex shows that variability in the angle between the naso-meatal plane and the occlusal plane is comparable with variability found in other angles connecting facial landmarks, i.e., it is neither excessively variable nor markedly stable.

The differences due to age may be explained in the light of known facts concerning cranial and facial growth. The planes (or better, lines) under consideration are determined by 4 points; 3 of them are facial points and 1 is cranial. Brodie (7) has shown that the occlusal plane—determined entirely by facial points—descends in a parallel fashion from the anterior cranial base, and that if a line from nasion through the center of sella turcica were prolonged to intersect the occlusal plane, the angle formed by that intersection would be, in the same individual, the same in adult life as it was in childhood. The naso-meatal line is determined by the anterior nasal spine, a facial point whose downward and forward descent from the anterior cranial base is not unlike the occlusal plane, and by porion, a cranial point. A serial examination of head-films reveals that porion grows downward and backward with respect to the cranial base; as a cranial point, however, it arrives at its adult position much earlier than do facial points. From this it may be seen that while the occlusal plane descends in growth as much at its posterior end as it does at its anterior end, the naso-meatal plane behaves differently. Due to the cessation of growth at its posterior end and the continued downward and forward movement of the anterior end, this plane tends to converge more and more with the occlusal plane until facial growth ceases. This phenomenon is illustrated in *fig. 1*.

[2] The coefficient of variation was not held to be a valid measure of dispersion in this situation, not only because angles measure relationship rather than size, but also because dividing the standard deviation by a mean approaching zero gives a coefficient of variation so large as to be entirely out of keeping with the facts.

It is evident from these data that if the clinician were to use the same points as did the author, an anterior convergence of about 5 degrees would more often yield a close approximation for the true occlusal plane than the classical parallel relation. If the commonly used soft tissue points were taken, the convergence would be more marked, since the alae are lower than the anterior nasal spine.

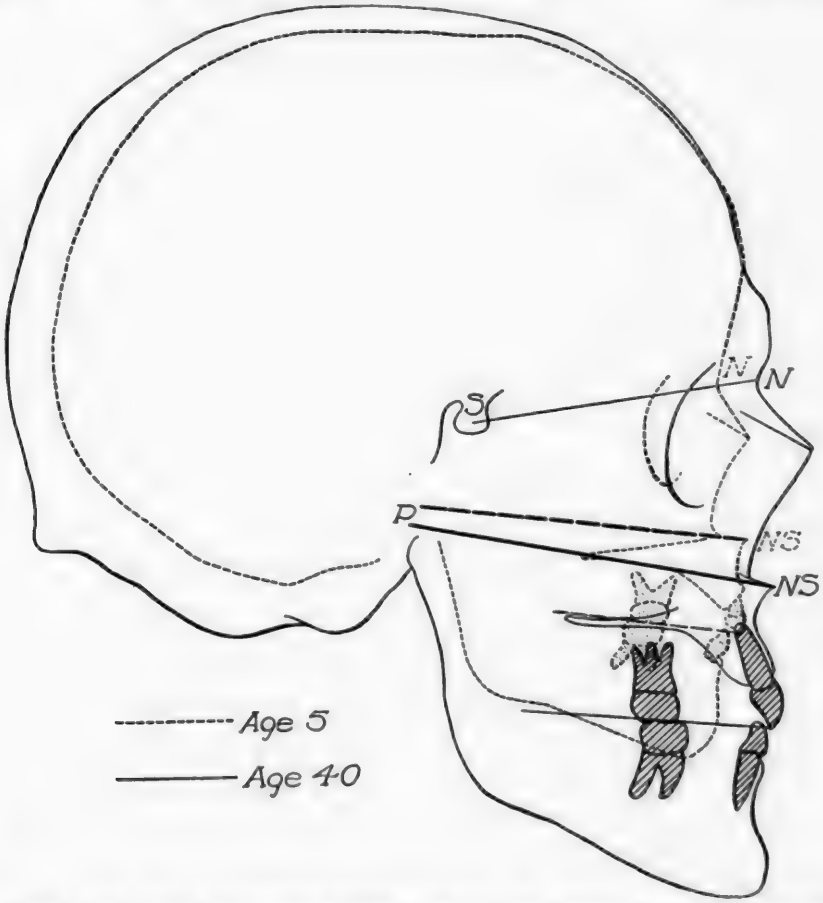

Fig. 1. Data from 2 individuals selected to show age changes in relation of occlusal plane to naso-meatal guide line. Note that occlusal planes of child and adult are parallel when tracings are superposed on sella-nasion, but that guide line has descended more anteriorly than posteriorly.

Because in actual practice the determination of the occlusal plane by this method is taken only as an approximation and final determination of it is governed by other factors, and since 5 degrees could hardly be estimated with any accuracy, any modification of traditional practice, while it might satisfy an academic point, seems scarcely worthwhile.

SUMMARY

1. It has been shown that in the child the occlusal plane more nearly parallels the naso-meatal line than it does in the adult; an explanation for this in terms of growth is offered.

2. The amount of variation in the relation of the occlusal plane to the naso-meatal line to the occlusal plane in the adult has been indicated.

The author wishes to thank Dr. Allan G. Brodie for permitting use in a different application data originally collected in his laboratory for a master's thesis.

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SODIUM HEXAMETAPHOSPHATE AS AN AID IN THE TREATMENT OF PERIODONTAL DISEASE[1]

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INTRODUCTION

Albright, Sulkowitch, and Chute (1) have described a non-surgical method for the removal of renal stones in which sodium hexametaphosphate was used as a solvent. This method gave the best results in cases in which the stones were of the phosphate type. It was this work which suggested to one of us (H. F.), that perhaps sodium hexametaphosphate would be effective in reducing the deposition of calculus on the teeth.

Sodium hexametaphosphate may be represented by the formula $Na_2(Na_4(PO_3)_6)$. Albright, Sulkowitch and Chute suggested the following reaction as being reponsible for the dissolution of phosphate stones:

$$(2Na^{+} + Na_4(PO_3)_6^{=}$$

$$Ca^{++}$$

$$(Ca_2(PO_3)_6)^{=}$$

indicating that a complex Ca metaphosphate is formed. If this reaction takes place and sodium hexametaphosphate dissolves calcium phosphate stones, it should be a good solvent for salivary calculus which has been shown by many investigators to be composed chiefly of calcium phosphate.

Sodium hexametaphosphate is also used industrially, as reported by Hatch and Rice, (2) to prevent calcium carbonate scale from depositing on the water side of condensers or heat exchangers, and in water conducting systems. Hatch and Rice (2) pointed out that sodium hexametaphosphate has two properties which are of considerable interest with respect to colloidal phenomena. It possesses definite surface active properties of its own at solid aqueous solution interfaces, and it has the ability of forming soluble complexes with numerous multivalent cations, thereby reducing the concentration to such a low value as practically to eliminate their agglomerating action towards various colloidal systems. They report that 1 to 5 parts per million of sodium hexametaphosphate will prevent the deposition of calcium carbonate from waters containing several hundred parts per million of calcium carbonate while the total amount necessary for actual calcium carbonate fixation would be 1,100 p.p.m. The deposition

[1] Presented at the 22nd General Meeting of the International Association for Dental Research, Chicago, March 18–19, 1944 (*J. D. Res.* **23**: 189, 1944). Received for publication June 17, 1944.

of calcium carbonate scale from high carbonate waters to which ammonia has been added can be prevented by the addition of small amounts of sodium hexametaphosphate. Rice and Partridge (3) showed that not only is scale formation reduced by the addition of small amounts of sodium hexametaphosphate but that scale already deposited is removed.

Because of the ability of sodium hexametaphosphate to prevent the precipitation of complex calcium salts from colloidal solutions and its ability to dissolve the salts already deposited, it appeared, therefore, that it might be effective in reducing calculus deposition on the teeth. Also because of its action as a wetting agent it appeared possible that it would also have a detergent action.

PROCEDURE

Before sodium hexametaphosphate could be used in the oral cavity, it was necessary to determine what effects it would have upon the teeth and filling materials contained in the teeth. The effect was determined by the following procedures: 1. Immersion of extracted teeth in sodium hexametaphosphate solutions of graded concentration, including a saturated solution, for intervals of 1 week to 6 months. 2. Immersion of extracted teeth containing silicate fillings in solutions of the same concentration for the same intervals of time. 3. Immersion of silicate material separately in duplicate concentrations for the same periods of time. 4. Because Albright, Sulkowitch and Chute (1) reported that sodium hexametaphosphate could be tolerated by the renal mucosa, it seemed reasonable that the oral mucosa would exhibit an even greater tolerance. Therefore, no experiments on mucosal effect were carried out, except that one of us (H. F.) used it in his own mouth for several months without noticeable effect on the mucosa.

From the above tests it was found that: 1. There were no visible changes in tooth structure. The enamel surface remained hard and smooth and was not deformed when scratched by a sharp steel instrument (*fig. 1*). 2. Silicate fillings in the immersed teeth underwent complete disintegration after a period of 3 to 5 weeks. The filling material was still retained in the cavity but was of a semi-fluid consistancy and could be washed away by running water or vigorous agitation of the solution. 3. Isolated masses of silicate filling disintegrated rapidly in all percentages, leaving a fine granular precipitate.

After demonstrating that tooth structure was not damaged by immersion in sodium hexametaphosphate of varying concentrations for periods up to 6 months we were ready to ascertain its effect upon calcular deposits.

Extracted teeth having moderate to heavy deposits of calculus on their surfaces were brushed well with an ordinary toothbrush and immersed in sodium hexametaphosphate solutions of graded concentrations including a saturated solution. The teeth were left undisturbed in the solutions for varying intervals of time.

RESULTS

Dilute solutions, in vitro, had very little effect. In saturated solutions the supragingival calculus was completely removed in 10 to 15 days. The dark,

hard subgingival calculus was partially removed in 10 to 15 days, but a longer interval was necessary to bring about complete dissolution. The organic matrix in which the calcium salts were deposited remained in situ unless disturbed by brushing or vigorous agitation of the solution. Much of the stain present on the teeth before immersion was easily removed by brushing after immersion for a few days.

From these observations it was believed that a saturated solution of sodium hexametaphosphate used as a mouth wash and a dentrifice might be effective in preventing the formation of calcular deposits on the teeth.

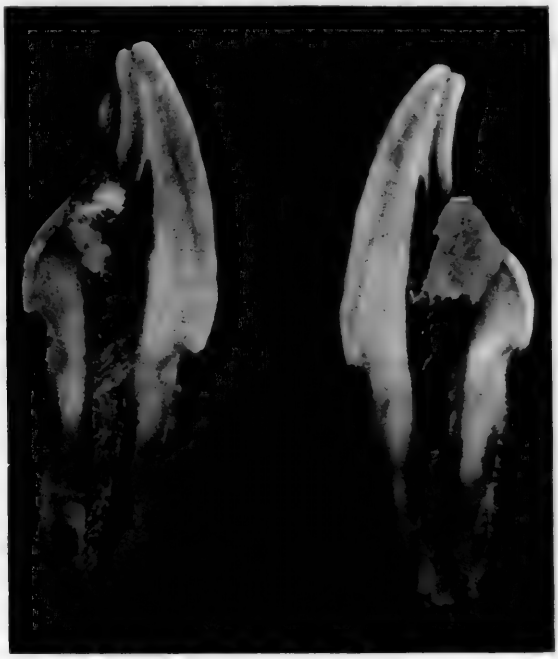

Fig. 1. Longitudinal section of tooth immersed in Sodium Hexametaphosphte for 6 months

To determine the effect of sodium hexametaphosphate on calculus formation a selected group of 10 patients undergoing periodontal treatment were used. All patients were known to acquire large amounts of calculus in a short period of time. Each patient was given a saturated solution of sodium hexametaphosphate to be used as a mouth wash and dentrifice once daily. They were directed to rinse the mouth with the solution every morning for 1 minute and follow this with their usual brushing procedure using sodium hexametaphosphate solution as a dentrifice. Patients were observed for intervals of a few weeks to a few months.

All patients using sodium hexametaphosphate daily ceased to have calcular deposits between appointments which were 1 week apart. The interval between

appointments was then increased to 1 month, and it was found that the same patients who had an appreciable amount of calculus deposited in 1 week now were free of deposit. The same results were obtained when the interval was extended to 6 weeks. There was also a reduction in the amount of stain accumulated during the same period. The teeth had an exceptionally clean appearance. If sodium hexametaphosphate as a mouth wash and dentrifice was discontinued for 1 to 2 weeks stain again accumulated and calculus was again deposited. In some patients calculus deposition could be prevented by using sodium hexametaphosphate in the prescribed manner every second day.

SUMMARY AND CONCLUSIONS

A preliminary report on the use of sodium hexametaphosphate as an aid in periodontal treatment indicates that:

1. Sodium hexametaphosphate in saturated solution does not have a deleterious effect on the teeth.
2. It does cause dissolution of silicate filling material at a rapid rate.
3. Supragingival calculus will be removed from extracted teeth in 10 to 15 days by a saturated solution, while dilute solutions have only slight effect.
4. Subgingival calculus will be partially removed in 10 to 15 days, but complete removal may take several days longer.
5. The use of a saturated solution of sodium hexametaphosphate as a mouth wash and a dentrifice prevents calculus deposition for periods up to 6 weeks and longer.
6. Because of its detergent action the accumulation of stain is also inhibited.

This should then be a valuable aid in the treatment of periodontal disease by preventing calculus and stain deposition.

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DIABETES IN RELATION TO CERTAIN ORAL AND SYSTEMIC PROBLEMS[1]

Part I: Clinical Study of Dental Caries, Tooth Eruption, Gingival Changes, Growth Phenomena, and Related Observations in Juveniles

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This study deals with certain oral and systemic problems in juvenile diabetics. It treats of tooth development, the incidence of tooth decay, the condition of the gingivae, and related observations.

The anterior pituitary gland is prominently involved in processes of bodily growth and development. Some of its hormones may also play a part in tooth development and eruption. Since the hypophysis has been implicated in the etiology of diabetes, the triangle of gross development, tooth development, and diabetes gives rise to questions of possible interrelationship (1).

The opinion that tooth decay is facilitated by factors of poor nutrition has been expressed frequently in the literature. For example, the fact that excessive amounts of sugar are present especially in the diets of some children has led certain observers to hold this agent responsible in some degree for dental caries. Boyd and Drain (2) and others studied the incidence of caries in diabetic children. They found a significant reduction in tooth decay in juveniles under good diabetic control and attributed the change to improved nutritional status. Their conclusions are in sharp contrast to the findings of those investigators who believe that in this problem the chemical composition of the diet is of secondary importance whereas its physical characteristics and local environmental factors exert the major influence in producing dental caries. One phase of the present study concerns itself with testing these conflicting hypotheses.

MATERIAL AND METHODS

Material for the present study was derived from examinations of children attending the camp of the New York Diabetes Association during the summer of 1941 and a few juveniles from the diabetes clinic of the Columbia University-Presbyterian Hospital Medical Center. In all there were 94 children.[2] The camp children were, for the most part, patients in clinics and hospitals of greater

[1] Aided by a grant from the Research Fund of the New York Diabetes Association, Inc. Presented at the 20th General Meeting of the International Association for Dental Research, March 14–15, 1942, New York City (*J. D. Res.*, **21**: 296, 1942). Received for publication June 5, 1944.

[2] 13 of the children were negroes and their findings were not computed so as to avoid another variable.

New York; a few were from the private practices of New York physicians. Their ages ranged from 4 to 19 years. The duration of diabetes was from 3 months to 14 years. Of the 94 examined, data on 81 white children were analyzed statistically. These subjects had diet prescriptions that were, on the whole, adequate for growth and maintenance of health. The daily diets ranged through the following qualitative categories: carbohydrates 120 to 300 gms.; proteins 45 to 120 gms.; and fats 30 to 145 gms. The lower levels were mainly those prescribed for the younger children; the higher levels for the older ones.

Clinical examination of oral structures was made in daylight with the aid of a flashlight when necessary. The teeth were examined with mouth mirrors and double-ended explorers. Data relative to the gingivae and oral mucosa was recorded. The following information was noted: *1.* Description of gingivae: color, texture, and pathological conditions (if present). The depth of the gingival crevice was also observed. *2.* Description of hard and soft deposits on the teeth and degree of calculus formation. *3.* The amount and color of tooth stains. *4.* The state of occlusion, using Angle's classification (3). *5.* State of eruption; number of permanent teeth present in the arch. A tooth was counted as erupted if 1 mm. or more was seen through the gingivae [criteria of Klein and Cody (4)]. The eruption state in the diabetic group was compared with the eruption state in the Hagerstown, Maryland, group, reported by these authors. Their data was compared with the eruption data of Cohen (5) for Minneapolis school children and Greenwald and East (6) for Chicago school children, and found to be comparable. *6.* Decayed surfaces, missing teeth and filled teeth (DMF). A pit or fissure was considered to be carious if the sharp point of an explorer could be engaged in the grooves or pits and stuck there. The Klein, Palmer and Knutson (7) DMF system of grading a mouth as to caries experience was used. Individual DMF rates for the diabetic group were compared with the normals established by these authors in Hagerstown, Maryland, and the *differences* were compared. It may be noted that in a comparison between the above group of normal children and a group of normal children in New York City (8) (both studies by Klein et al.), similar caries experiences were observed. *7.* Complete intra-oral x-ray examination of the teeth consisting of 14 regular and 7 bite-wing films was made in 40 of the diabetic cases. These were studied for the presence of cavities not seen clinically, but these additional cavities were not counted in the caries experience (DMF) cited above. They were also used to determine whether or not unerupted teeth were present in the jaws. An evaluation was made of "dental age" by amount of crown and roof formation, after the norms established by Logan and Kronfeld and modified by McCall and Schour (9). The alveolar bone was studied as to evidence of resorption of the crest, or poor calcification as evidenced by extremely radioluscent x-rays. *8.* Records of previous physical examinations, histories from referring doctors or clinics, and other pertinent data relating to the systemic phase. Data was also obtained as to date of birth; sex; height; previous illnesses; sexual maturity. Diabetic histories included age of onset; nature of treatment as to insulin dosage; caloric value of diets, distribution of protein, car-

bohydrate and fats in diet; presence or absence of concomitant systemic complications. The degree of diabetic control was classified as follows:

Type 1—uniformally good, practically from onset of disease. Infrequent mild lapses, if any.

Type 2—persistent glycosuria, poor adherence to diet. No acidosis.

Type 3—same as type 2 with frequent and/or recent acidosis.

The nutritional status of this group as it relates to vitamins A, B and C has been reported elsewhere (10).

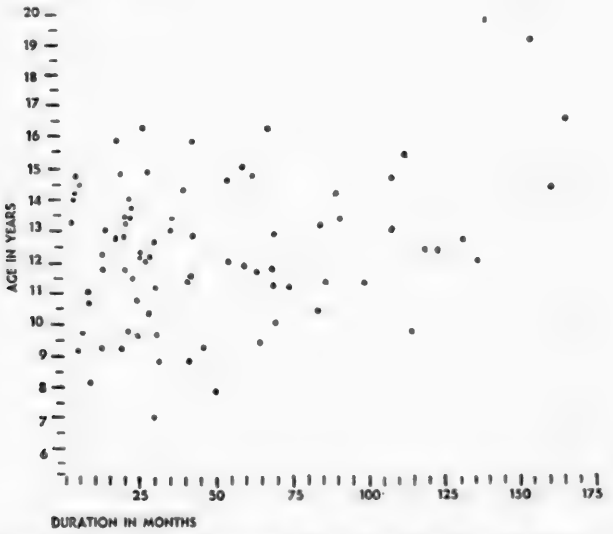

Fig. 1. Distribution of cases as to age and duration of diabetes

The data were analyzed by setting up a correlation graph for each of 2 sets of variables where the information permitted this method to be applied. The correlation charts were then bisected at the median for each variable, so that 4 quadrants were formed. The number of individuals falling into each quadrant should be equal if no relationship existed between the 2 variables under study. The numbers actually found were then compared with the expected number using the X^2 method. *Fig. 5* illustrates the method used.[3]

The distribution of cases as to age and duration of diabetes at examination is presented in *fig. 1*. The following variables were studied for relationships.

Height. On the assumption that over-activity of the anterior pituitary gland is responsible for diabetes, and that such over-activity stimulates growth, we tested height in relation to the duration of the disease (*fig. 2*); and in relation to age of onset, to determine whether early age of onset influences height to a different de-

[3] We wish to express our appreciation to Dr. John W. Fertig who suggested the above method for the analysis of these data.

gree than later onset in children of comparable age (*fig. 3*). According to published reports, juveniles having diabetes only a short time would be expected to fall into the category of "tall children" because of accelerated anterior pituitary gland function and because of the theory that long-time presence of the disease stunts growth. The age of onset factor was also studied because of stated hy-

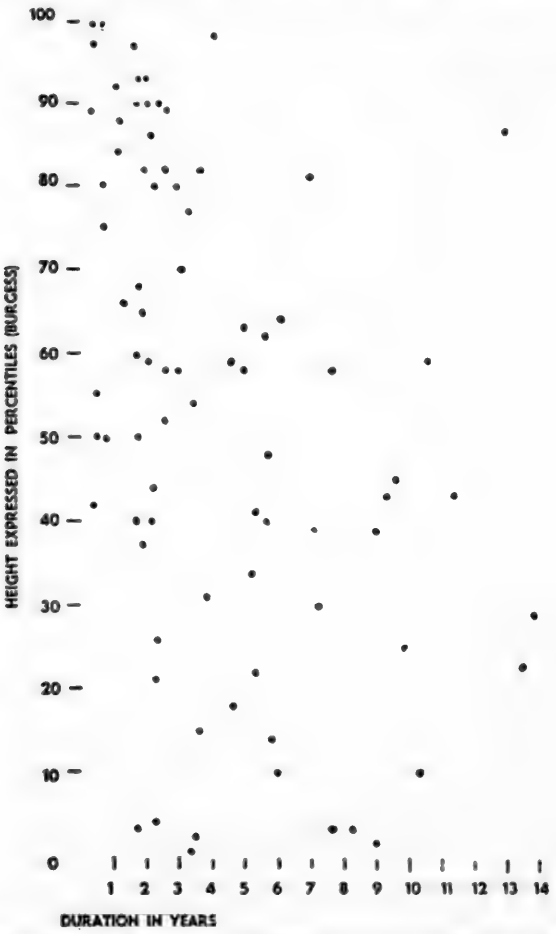

Fig. 2. Distribution of cases as to height for age and duration of diabetes

pothesis that diabetes in children starts mainly during growth spurts. For the purpose of this analysis, the diabetic children were classified according to height percentiles established by Burgess (11). Her method eliminates the age factor as a variable in computing height and thus provides a superior method of studying height.

Height was recorded in inches to one decimal place and was measured in stockinged feet. Age was recorded to the nearest month. To compute duration, the cases were divided into 3 groups: those with diabetes under 1 year; over 1 year, but under 3 years; and over 3 years at time of examination. This division was based on the report of Morrison and Bogan (12), who reported that at the onset of diabetes epiphyseal development is in advance of chronological age, whereas

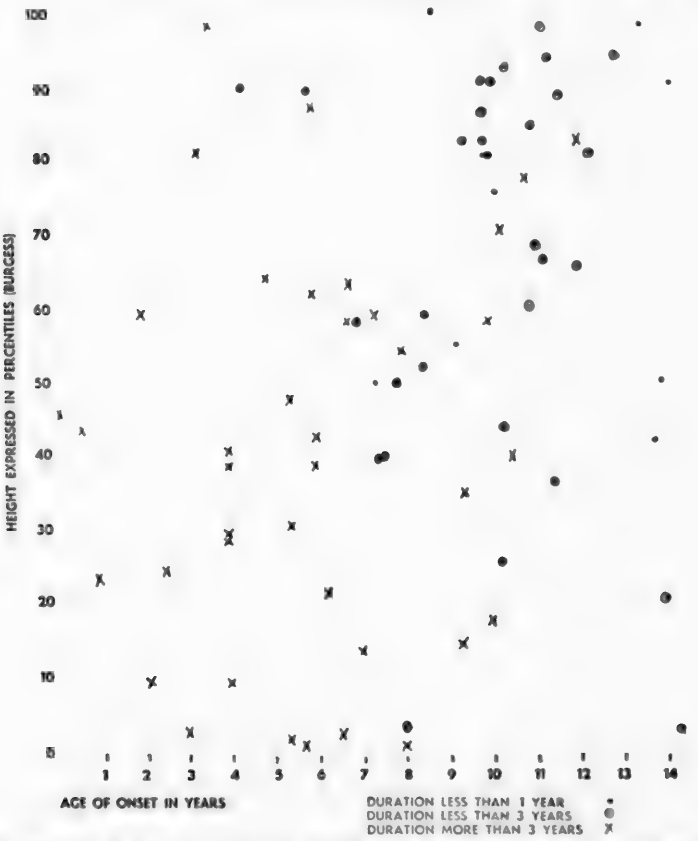

Fig. 3. This graph is arranged so as to test the effect of age of onset and duration on height percentiles of the diabetic children.

after a period of 3 years this development is delayed, the delay becoming more marked with time.

These procedures were followed throughout the study.

Eruption. This phase of the study was based on the theory that the eruption status of the permanent teeth may be an index of under or over activity of the anterior pituitary gland (13, 14). Thus, in cases of over-activity, resulting in tall

children, the eruption rate should also be accelerated. In cases of long duration, where under-activity of the anterior pituitary gland is said to be manifest, it would be reasonable to expect to find retarded eruption rate of the permanent teeth. The eruption rate was determined by computing percentages based on the data of Klein and Cody (4) for school children of Hagerstown, Maryland, and Greenwald and East (6) for school children of Chicago, Illinois. Analysis was made in relation to height and duration of the diabetes (*fig. 4*).

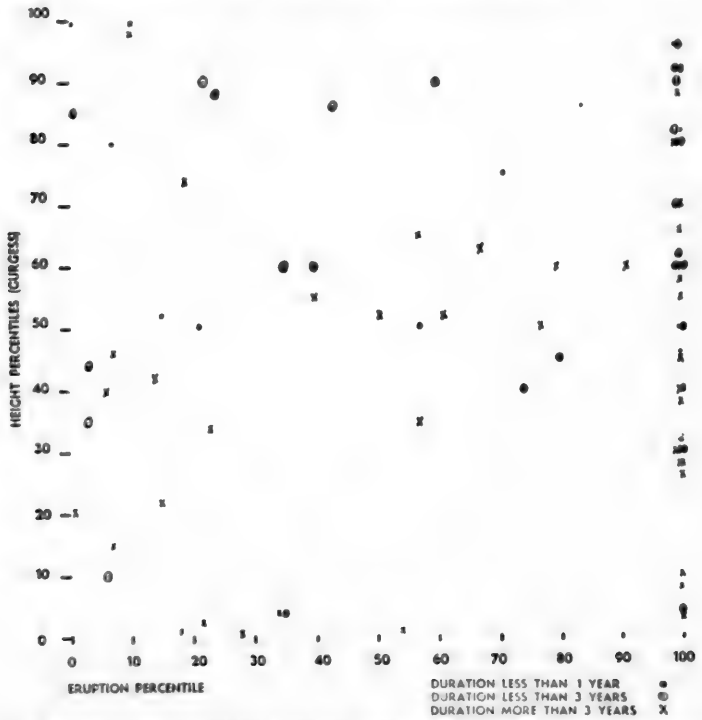

Fig. 4. Eruption percentiles are based on the number of permanent teeth erupted at the time of examination. Percentiles were calculated from averages of published data of Klein and Cody, and Greenwald and East.

Diabetic Control. The influence of the success of diabetic treatment on the factors under consideration is obvious. But because of our observation that children frequently become careless in adhering to the dietary regime as the disease progresses, and because an early age of onset is said to be associated with greater severity (thereby rendering control more difficult), diabetic control (Types 1, 2 and 3 as hereinbefore described) was tested with duration and age of onset.

Tooth Caries Experience. The implications here are chiefly nutritional in character and the deductions are largely by inference. The hypothesis has been

advanced that certain factors inherent in the diabetic state in children influence their nutritional status which in turn influences the amount of tooth decay. This is based on the assumption that a carefully regulated diet, a long duration, or early age of onset of the disease may provide a better opportunity for improved

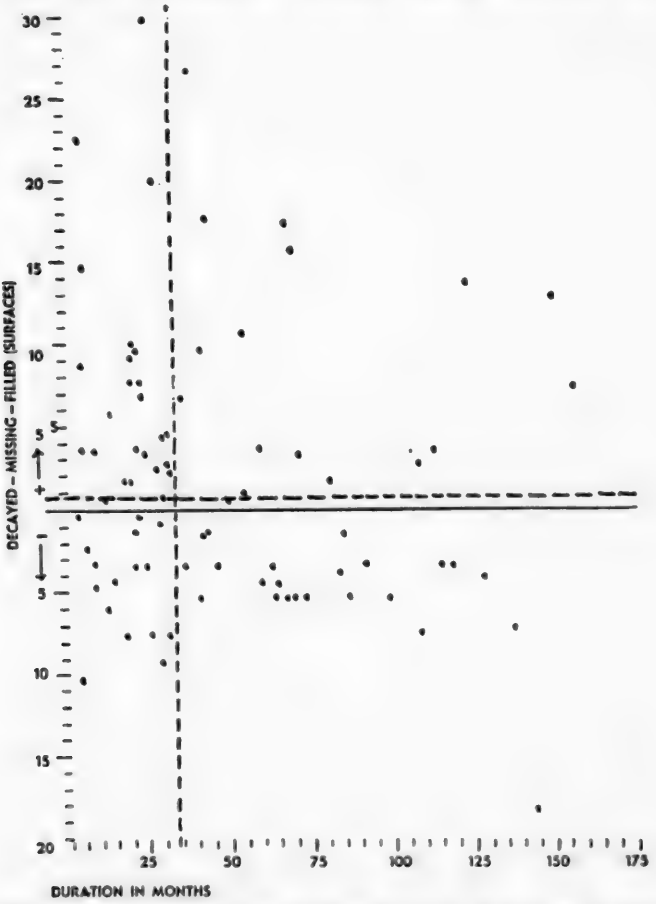

Fig. 5. The differences between caries prevalence in our group and a group of normal children in Hagerstown, Maryland. (The broken lines divide the cases into quartiles. The expected number of cases in each quartile is 20. The differences between the actual and expected numbers are insignificant.)

nutritional status than a short duration. On the other hand, degree of severity of the disease and laxness in adhering to a prescribed diet in connection with both these factors may mitigate against good control which in turn might react unfavorably in minimizing the amount of tooth decay. Tooth caries experience was analyzed in relation to duration of the diabetes (*figs. 5* and *6*), age of onset, and type of control.

Calculus Formation. Kent (15) reported in a group of diabetic children a marked tendency to calculus formation. Calculus formation was seen in a large number of the children examined in this study. These data were tested for a relationship between presence of salivary calculus and duration of diabetes or age of onset, in an effort to determine whether or not the diabetic state may be a contributory factor.

Color of Gingivae. Mouth examinations in most cases showed the gingivae to be violaceous in color instead of the pink color commonly seen in normal juveniles. The degree of change varied in intensity, the most pronounced being a deep violet.

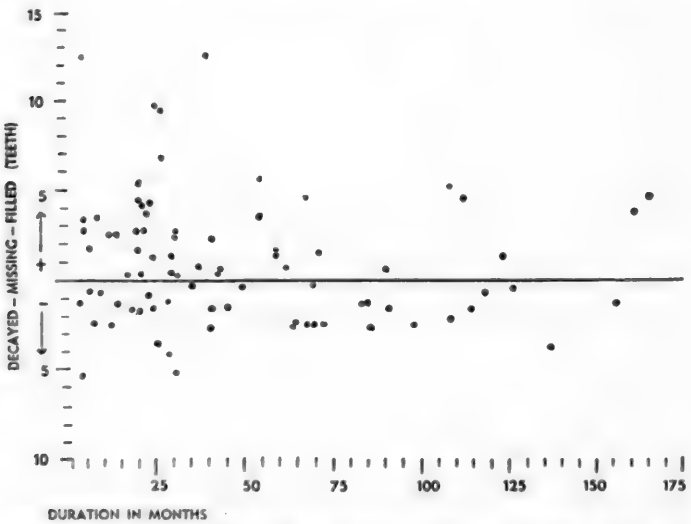

Fig. 6. Dental caries prevalence by teeth instead of surfaces as in Fig. 4

A possible etiological relationship was sought with some factor attributed to the diabetic state. Insulin dosage, age of onset, and duration of the diabetes were considered.

The degree of color change was graded from plus-minus to four plus, the former signifying little or no change, the latter indicating intense change. The insulin dosage was computed as to number of units in groups: 0 to 10 units, 10 to 20 units, etc. The protamin and regular insulin were totaled to obtain daily dosage.

Dosage of Insulin. Dosage was tested in relation to duration in order to ascertain whether or not time might have an ameliorating effect on the severity of the diabetes.

RESULTS OF CORRELATIONS

An analysis of these data shows the values for the 15 comparisons enumerated above to be insignificant. While we observed children showing characteristics

which have been described in the literature as causally associated with diabetes, our data negate previous generalizations (1, 2, 16). On the basis of our findings it cannot be said that any of these factors are definitely influenced by the diabetes per se.

RESULTS: OTHER FACTORS

X-ray examination, obtainable in 40 members of the group, disclosed in many instances cavities not seen in clinical examination. Five apparently caries-free cases showed evidence of cavities in the radiographs. There appeared to be no appreciable loss or decalcification of the alveolar bone. Further x-ray study showed that root development did not differ materially from the Logan and Kronfeld norms (9).

The oral hygiene was poor in about half the series. Gross observation of the gingivae revealed thickened gingivae with increased crevicular depth, in addition to the violaceous color previously mentioned. We observed varying degrees of the violaceous color in 92% of our cases. Of these, 40% were graded $+$; 29% were graded $++$ or $+++$; and 31% were graded $\pm$. There was an increase in the depth of the gingival crevice in 89% of the cases. 65% of the series had hard and soft deposits on the teeth. 26% had soft deposits without the presence of calculus. 75% of the cases had stained teeth. Of this number, 25% showed green stain; 16%, black stain; and 41%, orange stain. In 7% there was a combination of green and black, or green and orange, or black and orange.

Marginal gingivitis affecting the mouth generally was seen in 11% of the group. Localized areas of marginal inflammation were found in 19%. Histories of induced hemorrhage of the gingivae were obtained in 32%. About 2% showed spontaneous hemorrhage. 15% had spongy gums in either localized areas or generally. The gingivae of 85% of the cases were firm. In numerous instances of careless tooth-brushing habits, where a gingivitis might be expected to occur, there was no generalized gingivitis. This finding is in agreement with conclusions drawn by others (15, 17).

Malocclusion was common but not characteristic.

In a few cases of young adults attending Vanderbilt Clinic and under diabetic management since early childhood, several sets of radiographs, taken as the patient grew older, were obtainable. These, as well as gross examination, revealed advanced periodontoclasia accompanied by severe loss of alveolar bone. In one case, bone destruction had progressed to the extent that the floor of the antrum was resorbed in both halves of the upper jaw (*fig. 7*). In all instances, earlier x-rays taken during childhood failed to show extreme bone destruction.

Systemic observations included the factor of sexual precocity, based on the theory of accelerated anterior pituitary gland function. This group had *no case* of sexual precocity. There were a few adolescent females who, while normally mature anatomically, gave histories of delayed onset of menses. One 11 year old male was over-weight and appeared to fit into the picture of "Froehlich's syndrome," but may well have been a case of delayed pubescence. Ten children had hepatomegalia. Of these, 6 (girls) showed clinical evidence of vitamin B defi-

Fig. 7. S. B., female, white, age 22 years. Shows extreme resorption of alveolar bone. Note that bone forming floor of sinus has been resorbed in places. Onset of diabetes at age 15 years.

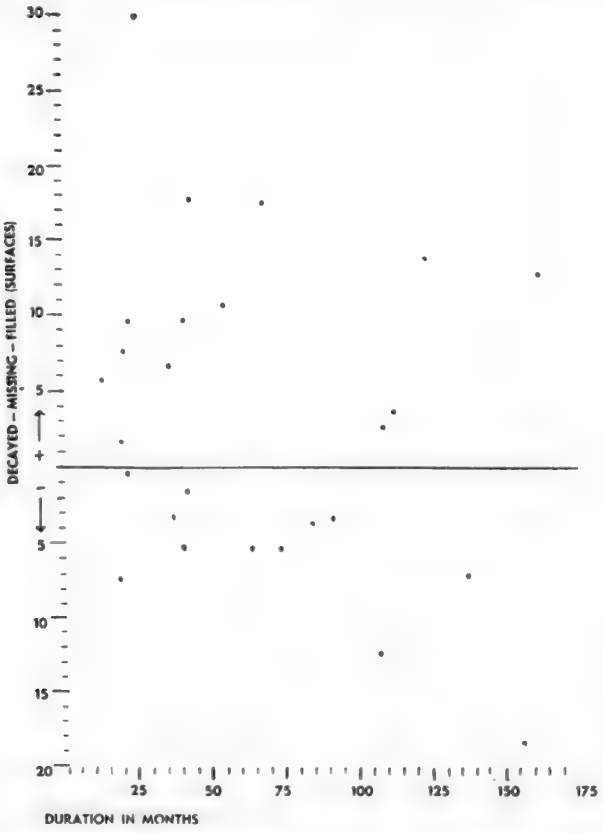

Fig. 8. Dental caries prevalence in a group within our diabetic group in which clinical signs of nutritional deficiencies were observed.

ciency. 22 others gave evidence clinically of vitamin B deficiency. *Fig. 8* shows the caries experience in these cases, indicating no apparent relationship to the B deficiency. A comparison of the systemic findings in males and females revealed no material difference except for the 6 girls who had both hepatomegalia and clinical signs of vitamin B deficiency. There were no important findings relative to previous illnesses, nor systemic complications other than the ones already mentioned.

DISCUSSION

The chief purpose of this study was to attempt to determine whether or not the reported stigmata attributed to diabetes in juveniles could be correlated with development, tooth decay, or gingival status.

Dental and General Development. Schour and Van Dyke (13), and Downs (14) reported, respectively, retarded tooth eruption in hypophysectomized animals and accelerated eruption with hyperactive anterior pituitary function. Numerous studies have been made in an attempt to demonstrate the involvement of the anterior pituitary gland as a factor in the production of diabetes (18, 19, 20, 21, 22, 23). The question then presents itself: are the disturbances of the anterior pituitary gland which are believed to be a causative influence in diabetes likewise implicated in general and dental development in diabetic juveniles?

White (1) stated that the development of the teeth of diabetic children parallels other forms of growth. She stated that the dentition is in advance of chronological age at the onset of the diabetes and retarded after establishment of the disease. She also reported that some juveniles were overheight for their ages at the onset of the disease and that some had precocious puberty. She also noted that the onset of the diabetes tended to coincide with periods of greatest growth.

In the present study, the eruption state of the teeth was compared with normals and variations noted. An attempt was made to correlate these variations with factors of height and duration. These correlations were found to be insignificant. Our series consisted of some children who were exceptionally tall at the onset of the disease and some who were exceptionally short after the diabetes had been present for several years. However, when comparison was made with the Burgess height charts for normals the correlations were found to be insignificant. Brown and Thompson (24), also using Burgess standards, found that the heights in their diabetic juvenile series did not vary significantly from the normal. (It should be noted here that in our study as well as in that of Brown and Thompson, heights at the onset of the disease were not available in all cases.)

The presence of precocious puberty in diabetics, noted by White, was not seen in a single case of our group. Neither did our study confirm the contention of this writer that "the ages of greatest incidence of diabetes are the same as those in which the greatest spurts of growth occur."

Our data is also at variance with the statement made by White that dental development in diabetic children parallels other forms of growth. We considered the number of erupted teeth as one of the criteria of dental development. The amount of crown and root formation, as disclosed by x-rays, constituted another. Our findings in this regard are in agreement with those of Brauer and Bahador

(25) who studied tooth development from radiographs. They stated that there was no significant correlation between the chronological age, onset of diabetes, duration, bone age, and tooth development.

Eliminating the diabetes factor for the moment and considering the problem of general development in the light of other gross metabolic disorders which may result in growth disturbances or sex abnormalities, there is evidence in advancement of the hypothesis that tooth development does not necessarily parallel other forms of growth. Jones, Larsen and Pritchard (26) indicated that bone development and tooth development may be independent of each other. Ziskin, Salmon, and Applebaum (27), in a study of new-born thyro-parathyroidectomized rats, found that while skeletal development terminated at about age 14 days and thereafter maintained a plateau at this level, the teeth continued to develop (at a subnormal rate) throughout the experiment of 70 days. Clarke and Smith (28) reported other severe deprivation states during which the teeth continued to develop even at the expense of bone. In a study involving a group of children in whom manifestations of puberty were seen at 2 to 4 years of age, the bone development was considered to be advanced from 2 to 10 years whereas the dental growth was in agreement with the chronological age (Ziskin [29]). In another study dealing with "pseudo-Froelichs" whose puberty was delayed up to several years beyond the expected "normal," dental development was found to be normal (Ziskin [29]).

Dental Caries. It has been stated (30) that prior to the insulin era, tooth decay was rampant in diabetics. These reports attempted to link the diabetic state with tooth decay, with special stress on cervical caries. Boyd and Drain (2) and Boyd (31) found that dentin caries in children was arrested following control of diabetes. They stated that in controlled cases, over a period of years, there was a marked reduction in the incidence of new lesions. The improved nutritional status was held responsible.

Rutledge (32) found little tooth decay present in a group of 18 juvenile diabetics. Kent (15), Rudy and Cohen (33)[4] reported caries frequency in diabetic children to be comparatively low. Our findings are not in agreement with those cited above. While there were several instances of caries-free mouths, the group as a whole showed characteristics similar to seemingly normal, non-diabetic juvenile groups. In an attempt to charge nutritional factors with the variation in caries experience, we compared diabetic control with the amount of tooth decay and were unable to find a correlation. We compared duration of disease and age of onset with both DMF teeth and DMF surfaces and found no data to support the idea that diabetes either through nutritional or other factors altered the caries incidence. Kniesner, Mann and Spies (34) found no relationship between vitamin B complex deficiency and tooth decay. Our group of "B" deficient children was similarly negative in this respect. Block (35) examined a large number of juveniles with xeropthalmia. He reported no increase in tooth decay. Lindstrom (36), Grandison, Stott and Cruickshank (37) and Sandburg and Dagulf

[4] These authors are now of the opinion (personal communication) that the caries incidence in diabetic children does not differ materially from normals.

(38) found no difference in caries frequency in children and adults deficient in vitamin C as compared with controls.

Further data supporting the opinion that nutritional inadequacy is not a determining factor in the etiology of dental caries is obtained from studies on primitive peoples (39). [See relevant review papers (40, 41, 42, 43).]

Gingival Status. Gross examination of the gingivae showed the prevalence of a violaceous color, in varying degrees of intensity, instead of the pink color commonly associated with the gums of children. Insulin dosage, age of onset, and duration of the diabetes were considered as etiological possibilities, with negative results. Nevertheless, owing to the consistent and striking nature of the gum-color deviation, some element relating to the diabetes, as yet obscure, probably acts as an influential causative factor. In addition, it was noted that the gingivae were thicker than normal, with increased crevicular depth. 85% of the cases showed firm gingivae despite the fact that gingivitis would ordinarily be expected in a large number because of careless toothbrushing habits.

SUMMARY

Ninety-four diabetic juveniles, 4 to 19 years of age, were studied, the data on 81 of the number being analyzed by statistical methods. The duration of the diabetes was 3 months to 14 years. Data directed at ascertaining the relationship between diabetes and growth and development as a whole, and the teeth in particular, yielded insignificant correlations. They point to the conclusion that precocious general, sex, and dental development in juveniles cannot be linked *per se* to the diabetic state.

In contradiction to previously published reports, no significant reduction was found in the incidence of tooth decay in diabetic children. This finding minimizes the theory that caries of the teeth may be controlled by nutritional means via the general metabolism.

In most cases the gingivae were violaceous in color, thickened, and of increased crevicular depth. They showed important changes of a beneficial nature. These changes were associated with hyperplasia, hyperkeratinization, and increased glycogen deposits in the epithelium. They are discussed in detail in the histological study which is Part 2 of this report (44).

The authors wish to thank Drs. Chas. M. Chayes and Halsey Raffman for their assistance in oral examinations.

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REESTABLISHMENT OF TRANSSEPTAL FIBERS FOLLOWING EXTRACTION[1]

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The transseptal fibers of the periodontal membrane are those ligament-like fibers which run between the proximal cervical aspects of the roots of approximating teeth, passing just oral to the crest of the interdental septum. Since this group of fibers was first named by Black in 1886, they have generally been considered as having the function of maintaining the mesiodistal relationship between neighboring teeth (e.g., Kronfeld, 1939).

With respect to this function there has been speculation and some experimentation concerning what happens when these fibers are damaged or destroyed. Waugh (1904) stated that "as a result of the immediate method of separating teeth, the fibers of the transseptal group are snapped, resulting in degeneration of the membrane". He recommended therapy to encourage repair of the damage, but believed that "complete restoration of the original conditions" was not to be expected.

Waldron (1942) wrote: "It is the opinion of Oppenheim that when molar teeth are being moved distally and large spaces appear between the teeth, anterior to the teeth which we are striving to move, then the teeth are being moved too rapidly, and the transseptal fibers are being broken. This breaking of the fibers interferes with their function of maintaining the mesiodistal relationship between neighboring teeth, and thus we lose the benefit of the action of the transseptal fibers during the process of retention."

Gottlieb and Orban (1938) referred to the shifting of this group of fibers apically to keep pace with resorption of the interdental septum, thereby demonstrating that reorganization can and does take place in this fiber-group. That regeneration and orderly reorganization might be expected here was also indicated by the results of the experiments of Beckwith and Williams (1928) and of Axhausen (1937). Although neither of these experiments dealt with the transseptal group of fibers, both showed that other fibers of the periodontal membrane, after injury and partial destruction, were regenerated to reproduce their original arrangement rather than merely scar tissue.

This paper is a report on the reestablishment of the transseptal fibers after they had been broken by the extraction of a tooth.

[1] Read at the 22nd General Meeting of the International Association for Dental Research, Chicago, March 18–19, 1944. (*J. D. Res.* **23**: 207, 1944.) Received for publication June 30, 1944.

METHOD

Our experimental animals were female macaque monkeys approximately 3 years old. In each animal a second deciduous molar was extracted, in macaque number 3 a lower, in number 4 an upper. Following the extraction an orthodontic appliance consisting of cast bands and horizontal buccal tube and sliding shaft was cemented on the first deciduous and first permanent molars. The appliances were activated by elastics stretched over hooks on the molar bands.

Fig. 1. Mesiodistal section through lower left molar region of macaque number 3. Section passes through "gubernacular" foramen of second premolar. Orig. mag. × 6.

Fig. 2. Reorganized transseptal fibers in extraction space between lower left first deciduous and first permanent molars of macaque number 3. Orig. mag. × 24.

Fig. 3. Reorganized transseptal fibers in extraction space between upper left first deciduous and first permanent molars of macaque number 4. Orig. mag. × 18.5.

Fig. 4. Normal transseptal fibers between upper left deciduous canine and first deciduous molar and reorganized transseptal fibers in extraction space distal to first deciduous molar of macaque number 4. Orig. mag. × 18.5.

In animal number 3 the first such appliance was cemented in place 3 weeks after extraction of the deciduous molar. This appliance was worn for more than 2 weeks, when it was pulled off by the animal and lost. A new appliance was inserted and activated about 2 weeks later (8 weeks after the extraction). The elastics for this appliance were changed thrice weekly for 6 weeks. The animal was sacrificed 14 weeks after the extraction.

In animal number 4 the appliance was worn during the last 3 weeks of the 5 weeks following extraction of the second deciduous molar, with weekly change of the elastics.

When the animals were sacrificed, blocks including the teeth of experimental regions and blocks of the opposite normal regions (used as controls) were sawed from the jaws and fixed in 10% neutral formol. These blocks were decalcified in 5% nitric acid and embedded in low viscosity nitrocellulose. Sections were stained in Lillie's acid hemalum and eosin.

OBSERVATIONS

Examination of such sections (*figs. 1–4*) shows that the regenerated gingival tissue formed in the extraction space includes in it a group of fibers which strongly resemble normal transseptal fibers. This group is inserted in the distal cervical

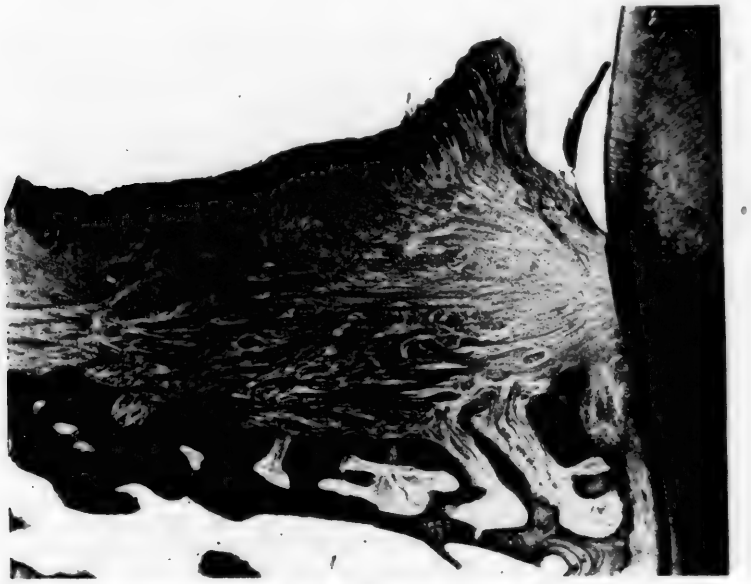

Fig. 5. Reorganized transseptal fibers in extraction space mesial to lower right third molar of man, aged 28, whose first and second molars had been extracted. Orig. mag. × 20.5.

cementum of the first deciduous molar and runs as a dense ligament-like group of nearly parallel collagenous fibers to insert in the mesial cervical cementum of the first permanent molar.

While this fibrous band is not quite as wide nor quite as regularly arranged as the normal transseptal fibers, it does form a tract which completely and continuously bridges the extraction space (*figs. 2, 3,* and *4*). It must have a functional relationship closely approximating that of the normal transseptal fibers.

Examination of mesiodistal sections through human teeth which adjoin extraction spaces shows similar bands of nearly parallel collagenous fibers bridging the extraction spaces (*fig. 5*).

For the human teeth we have no knowledge as to the length of time intervening

between extraction and preparation of the specimens. In macque number 4, however, the process of reorganization was completed within a period of 5 weeks. For the last 3 weeks of that time the teeth on either side of the extraction space were subjected to a continuous tractive force of about 70 grams by means of the orthodontic appliance. Hence the reorganized collagenous fibers have achieved their orderly, nearly parallel arrangement and close resemblance to normal transseptal fibers in spite of this orthodontic force.

In view of this capacity of the gingival connective tissue to regenerate in such orderly, apparently functional fashion, is it not probable that the ill effects of damage to the transseptal fibers have been overemphasized? The extensive tearing of the transseptal fibers found by Urban, Beisler and Skillen (1931) when they separated dogs' incisors forcibly would, in view of our findings, probably have been only temporary damage. We could expect such damaged tissues to become repaired in a relatively short time, to reestablish their original orderly arrangement with resumption of essentially normal function.

CONCLUSIONS

The authors certainly do not wish to recommend the employment of unnecessarily destructive clinical procedures. They do, however, conclude that regenerative processes will, in all probability, tend to restore a reasonable reproduction of original functional conditions following even severe injuries to the transseptal fibers.

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THE PAIN THRESHOLD RAISING EFFECTS OF AMPHETAMINE[1]

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It has been known for some time, though not generally recognized, that various sympathomimetic drugs manifest an analgesic effect. The literature on this subject has been reviewed by Ivy, Goetzl, Burrill, and Harris (1). From studies on mice and dogs (2, 3) it was learned that amphetamine possessed such analgesic and other effects that it might be useful clinically in the control of pain. As preliminary to other clinical investigations, the present experimental study on human subjects was undertaken for the purpose of comparing the pain threshold raising effects of the isomeres of amphetamine with like effects of other well-known drugs.

METHODS

This study was carried out with the cooperation of several groups of normal adult subjects, totalling 34 individuals, all students in the Northwestern University Dental School and working in the dental clinic during the experiments. The method of determining pain threshold was a modification of that devised for use on dogs as described by Goetzl, Burrill and Ivy (4). The mechanism of the pain threshold measuring apparatus consisted of 2 coils. The primary coil was mounted on a pivoted arm so as to swing in a plane parallel to its windings, and was rotated in the plane by an electric phonograph motor. The secondary coil was mounted parallel to the primary and on a second arm arranged to swing in a plane parallel with and just above the plane of motion of the primary coil. When the primary coil passed close to the secondary coil there was generated in the secondary coil a sudden peak of alternating current. The peak voltage generated in the secondary coil was varied by moving the secondary coil towards or away from the path of the primary coil. Between peaks no current could be detected in the secondary coil by means of a standard oscilloscope. The arm carrying the secondary coil passed over a scale calibrated to show the voltage of the peak current delivered with the secondary coil in any position.

One side of the secondary coil was connected by a gold electrode to a metal filling in a subject's tooth. The same tooth and filling were used in all tests for any one subject. The other side of the secondary coil was connected to a salt water bath into which the subject placed one hand.

It was found that a sensation of pain was the only definite sensation that could

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be produced in a tooth, though for most subjects there was an indefinite, indescribable sensation of very low intensity just before the painful level of stimulus was reached. The lowest voltage of current which just sufficed to produce a painful sensation was accepted as the measure of the pain threshold for the subject at the time. At each determination, the threshold value accepted for the subject was the lowest voltage which produced a painful sensation 3 times in succession. It was found that when interfering factors, such as a heavy lunch, a cold, or previous administration of drugs, were avoided, each subject at the same time of day responded initially at approximately the same voltage. Over several months' time, however, this initial threshold tended to rise gradually.

The experiments were carried out in the afternoon. Preadministration readings were taken at 1:30 and 2:00 P.M. The 1:30 readings were variable and were considered only as recalling to the subjects the intensity of sensation to be reached as the end point. The 2:00 o'clock readings were constant and were accepted as the true initial values. The drug used was administered orally at 2:00 P.M., and readings were taken thereafter every half hour for 2 hours. The results were recorded as actual voltages, and the mean of the differences of the 4 post-administration values from the initial or preadministration value was accepted as indicating the analgesic effect of the drug. To check the values obtained, *placebos* were substituted for the drugs at frequent intervals. The testing machine was so placed that the subject could not know the voltage applied to his tooth; and in carrying out the tests care was taken that no constant pattern of voltage changes was followed. The subject concentrated on holding the electrode against his tooth and on the resultant sensations. Each signalled vocally when the painful level of stimulus was reached. The subjects were faithful in reporting for the tests and it is a pleasure to thank them for their assistance.

RESULTS

During the course of the experiment, 13 afternoons were devoted to making control determinations with placebos. It was found that in general the voltages of the pain thresholds tended to decrease during the afternoon in these determinations. This is reflected by a mean drop of 0.005 volts in the control determinations. In other words, as the afternoon passed, the subjects' pain sensitivity increased. However, if there was any marked disturbance in the routine of the subject's work in the dental clinic, such as difficulty in placing a foil filling or unusually good results in a dental operation, the threshold values increased and appeared to do so in proportion to the amount the subject was disturbed or elated. It was observed that running upstairs invariably raised the thresholds, as did any marked disturbance in the room where the tests were carried on. The changes in control values produced by these factors were not great in any case, but were sufficient to attract our attention.

In the control determinations, the greatest mean increase in threshold values for the several groups was 0.026 volts. The greatest mean decrease for the several groups was −0.035 volts. Of the 118 individual control determinations, only 13 showed a rise of threshold of more than 0.04 volts. Changes of this magnitude produced by a drug therefore are to be considered meaningless.

Table I shows the drugs used, the dosage, the number of subjects showing a rise, a drop, or no change of threshold, and the means of all *changes of threshold values*, expressed in volts. All values obtained on control determinations are included. Values obtained during known times of stress or the like and known to be incorrect for such reason are excluded from the figures showing the effects of drugs.

TABLE I

DRUG	DOSE	THRESHOLD CHANGES			MEAN CHANGE
		No. up	No. down	No. unchanged	
					volts
Placebos		42	51	25	−0.005
Aspirin	10 gr.	15	5	4	+0.059
Phenacetin	6 gr.	10	4	3	0.013
Codeine	0.5 gr.	11	7	0	0.022
Codeine and	0.5 gr.				
dextro-amphetamine	10 mg.	6	2	1	0.021
Aspirin,	7.5 gr.				
acetophenetedin &	7.5 gr.				
r-amphetamine	7.5 mg.	10	3	3	0.089
r-amphetamine	10 mg.	23	7	1	0.047
dextro-amphetamine	5 mg.	5	1	5	0.044
dextro-amphetamine	10 mg.	49	12	4	0.056
dextro-amphetamine	15 mg.	23	1	2	0.132
levo-amphetamine*	10 mg.	17	2	0	0.064
levo-amphetamine	10 mg.	51	33	8	0.013
levo-amphetamine	15 mg.	8	0	1	0.116
levo-amphetamine	20 mg.	6	1	1	0.062
dextro-amphetamine &	5 mg.				
levo-amphetamine	15 mg.	18	2	0	0.080

* First administration of the drug.

From the data, it appears that there is a discrepancy between the dosage of levo-amphetamine and the threshold changes. The reason for the peculiarity is that the analgesic effect of levo-amphetamine appears to drop rapidly on successive administrations. Most of the tests on levo-amphetamine followed closely after earlier administrations of the same drug or after r-amphetamine which, because of its content of the levo isomere, has somewhat the same effect. Initial administrations of 10 mg. levo-amphetamine gave the results noted as of the first administration.

EFFECTS OF SUCCESSIVE ADMINISTRATION OF DEXTRO- AND LEVO-AMPHETAMINE

To compare the effects of dextro- and levo-amphetamine, they were given alternately in 10 mg. doses. The results were as shown in table II.

Each isomere was then given repeatedly, with the results shown in tables III and IV.

From these results it appears that the analgesic effect of levo-amphetamine decreases rapidly with successive administrations, and that one administration

TABLE II

DATE	ISOMERE OF AMPHETAMINE, 10 MG.	CHANGES IN THRESHOLD			MEAN CHANGE
		No. up	No. down	No. unchanged	
					volts
11–26	levo	8	2	0	0.059
11–29	dextro	7	2	1	0.045
12–1	levo	3	5	3	−0.011
12–3	dextro	9	1	1	0.047
12–6	levo	3	4	0	0.016
12–8	dextro	6	2	1	0.033

TABLE III

Successive administrations of levo-amphetamine, 10 mg. which were then followed by dextro-amphetamine

DATE	CHANGES IN THRESHOLD			MEAN CHANGES
	No. up	No. down	No. unchanged	
				volts
2–11	9	0	0	0.070
2–16	7	3	0	0.007
2–18	5	4	1	0.005
2–23	2	2	1	0.007
2–25	4	4	2	0.010
3–1	4	5	0	0.035
	dextro-amphetamine, 10 mg.			
3–3–3	7	1	0	0.074

alters the effect of another as much as 7 days later. This apparently is not true of dextro-amphetamine, the effect of which decreases slightly, if at all, on successive administrations. Neither isomere has any effect on later administrations of the other.

One in 5 or 6 administrations of dextro-amphetamine in the dose used produced slight central stimulation and wakefulness. The stimulation lasted about 2.5 hours and the wakefulness about 10 hours. Levo-amphetamine produced central stimulation only rarely but occasionally produced changes in heart rate or a slight

depression. In general, a subject who was stimulated by dextro-amphetamine was not affected by levo-amphetamine, and one who noticed effects after levo-amphetamine was not stimulated by dextro-amphetamine.

For comparison with the above values, the mean threshold change produced by 16 mg. morphine sulfate given subcutaneously to 16 normal human subjects was found to be 0.22 volts. Most of the subjects receiving morphine were depressed and nauseated (Ivy, Goetzl and Burrill, results to be published).

As tested by the present method it appears that both dextro- and levo-amphetamine are very definitely analgesic in doses of 15 mg., not only in comparison with aspirin and the like, but even in comparison with morphine and codeine. It is possible that the diminishing effect of the levo isomere of amphetamine contraindicates its use except for single administrations. So far as is known, the dextro isomere can be used repeatedly without marked loss of effect. Whether this will be true with intensive use or over long periods is not known.

TABLE IV

Successive administrations of dextro-amphetamine, which were then followed by levo-amphetamine

DATE	DOSE	CHANGES IN THRESHOLD			MEAN CHANGE
		No. up	No. down	No. unchanged	
	mg.				*volts*
5-3	10	7	1	1	0.098
5-5	10	6	1	0	0.049
5-12	15	10	0	0	0.146
5-17	15	7	0	0	0.182
5-19	15	6	1	2	0.087
levo-amphetamine					
5-21	15	8	0	1	0.116

DISCUSSION

Underlying any study of pain thresholds is the assumption that the stimulus used produces pain. So far as is known, the tooth pulp is not capable of any definite sensation other than that of pain. It is known that heat and cold cause a tooth to hurt and that sensations of temperature change are not felt as such. Likewise, a touch on or injury to the pulp of a tooth produces no sensation other than that of pain. Cutting the dentin of a tooth produces only a sensation of pain. If the stimuli are of very low intensity, the sensation may not be definitely painful, but neither is it identifiable as any other specific sensation. These observations are common among dentists and their patients. In general, either a tooth is sensation free or it hurts.

The subjects of the present experiment reported that as the voltage of the current applied to a tooth was slowly increased, there was first an unidentifiable, indefinite, non-painful sensation of very low intensity. This non-painful sensation is described by some as being analogous to a non-painful contact, or

"tap", or prick. Then with a small increment of voltage there was a slight pang, like a momentary toothache, at each peak of the current. If a current of too high voltage was accidentally applied, there was a general reaction, the subject exclaiming or jumping. This was true of all subjects but one. This exceptional subject did not feel definite pain until the current had reached a comparatively high voltage. The same subject reported that he experienced no discomfort when having cavities cut in his teeth. It is our belief that this subject felt the same sensations as the other subjects, but interpreted them differently. At a voltage level corresponding to the threshold levels of the other subjects he experienced a definite, though reportedly not painful, sensation. His thresholds based on this level of sensation varied in the same way as did the thresholds of the other subjects when various drugs were used. In studying the reactions of this subject, the authors found that by regarding objectively their own sensations caused by application of current to a tooth, they could alter the apparent nature of the ordinarily painful sensations in such a way that they were no longer painful, though not pleasant. It is known, furthermore, that some people can achieve this effect with the discomfort produced by dental operations. This would not appear to argue against the validity of measurements based on electrically produced tooth pain in testing analgesia, so much as it would seem to indicate that the intensity and possibly the nature of some painful sensations depend not only on the stimulus but also on the cortical processes involved in interpreting the sensation. It is possible that such changes in interpretation account, at least in part, for the analgesia produced by drugs.

Early in the work on human subjects it was found that at the voltage level which produced the first perceptible, though not painful, sensation, the subjects responded more uniformly than at the higher levels, possibly because at the higher levels some interpretation of the sensation was required—i.e., an inquiry by each as to whether the sensation was painful. But at the lower levels of voltage without painful sensation the ordinarily analgesic drugs produced only small change in threshold values, while at the higher levels of voltage with painful sensation, the same doses of the same drugs produced in the same subjects marked changes in the threshold, as indicated by changes in the voltage which produced the selected intensity of sensation.

At and slightly above the point at which the electrically produced sensation first became painful in each subject, it was found that the subject could perceive differences in intensity of sensations produced by changes in the current amounting to 0.0125 to 0.025 volts. The change in voltage required to produce a change in intensity of sensation was fairly constant for each individual and did not appear to depend on the absolute voltage level. In other words, subjects responding at 1.50 volts perceived changes in current of about the same magnitude as did subjects responding at 0.50 volts. For this reason, the actual changes in voltage, rather than the percentage changes in voltage, were accepted as indicating the changes in thresholds.

After the administration of certain drugs, such as levo-amphetamine in 15 mg.

doses, some subjects reported that the sensations were dulled and that it was difficult to tell when they became painful. At such times the ability of the subjects to perceive minor changes in stimulus was decreased. The spread between the first non-painful sensation and the first painful sensation was increased. Without drugs the painful sensation appeared 0.1 to 0.3 volts higher than the first sensation. With the drugs the difference was sometimes as great as 0.6 to 0.8 volts. The drugs did not raise the threshold of nonpainful sensation as much as they did the threshold of painful sensation.

The reason for the diminishing effect of levo-amphetamine on repeated administration is not apparent. Such an effect might be produced if the threshold raising effect of one administration had not worn off by the time of the next one, but in general the thresholds had returned to or toward their initial levels by the end of each 2 hour test period, and at succeeding administrations the pre-administration thresholds were not enough higher than before to account for the difference. There was a general tendency for the initial thresholds to rise over a long period, with some irregularities, but when the post-administration thresholds failed to rise above pre-administration levels, the pre-administration levels were low as often as they were high. The amount of change from pre-administration to post-administration level seemed to be independent of the actual value of the pre-administration threshold.

The threshold raising effect of dextro-amphetamine did not appear to diminish appreciably with repeated administration, though there may have been a slight change. Nor did either isomere appear to have any effect on the result of a later administration of the other isomere. No explanation is at hand for these peculiar differences.

It is not yet known to what extent the results of clinical use of the drugs tested will parallel the values obtained in the present study. Since the tests were made on teeth, and since the pain produced by the test current appears to be much the same as a mild toothache produced in any other way, there is reason to hope that certain of the drugs will prove useful for pre- and post-medication in dental practice. A drug that is effective against dental pain should help relieve certain other types of pain. Clinical experiments are now under way to determine the value of some of the tested drugs as pre-medicants to relieve the pain of dental operations.

SUMMARY AND CONCLUSIONS

1. The pain threshold raising effects of various drugs have been tested by means of an electric current applied to teeth through fillings. The values obtained are in terms of voltages and are compared.

2. It was found that the isomeres of amphetamine are relatively potent analgesics as tested by this method.

3. The threshold raising effect of levo-amphetamine diminishes markedly on repeated administration. This is not true of dextro-amphetamine. Neither dextro- nor levo-amphetamine has any influence on the effect of later administrations of the other isomere. No explanation of these results is available.

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EXPERIMENTAL PAPILLECTOMY[1,2]

Part II: Histological Study (Preliminary Report)

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INTRODUCTION

Transplantation of tooth germs or their elements has long been a subject of experimental investigation (1, 2, 3, 4, 5). Explants have also been grown in vitro (6, 7). The technics of Huggins (4) and Hahn (5) consisted, in part, of transplanting sections of the enamel organ and dental papilla in portions of the body other than the jaws. Glasstone grew whole tooth germs, as well as dissected portions of the enamel organ and dental papilla, in tissue culture.

These investigators (4, 5, 6, 7) have established an interrelation between the enamel organ and dental papilla. Their findings reveal that histodifferentiation of odontoblasts occurs only when ameloblasts are present. Thus the initial stimulus for formation of the dental tissues has been shown to be in the enamel epithelium. After differentiation of these two cell layers, the ameloblast apparently loses its ability to influence the formation of the dental papilla and odontoblasts. On transplantation of an isolated dental papilla with odontoblasts, it is capable of independent function in producing dentin. The absence of the enamel epithelium at this time does not impair the normal function of the papilla to form dentin. The ameloblasts, on the other hand, are incapable of independent function. If transplanted alone, they lose their ability to produce enamel. In the absence of the papilla, the enamel organ degenerates into squamous epithelium and forms small cysts.

Complete separation of the enamel organ from the already formed enamel is difficult and of questionable value. Tomes process of the ameloblast extends into the young enamel matrix[3]. It is not known whether Tomes process was removed with the enamel organ or remained as part of the young enamel matrix, in the experiments mentioned above. The dependence of the enamel organ on the activity of the dental papilla requires further examination, as does the function of the odontoblasts. Several unsolved problems present themselves. It is known that after formation of the first layer of dentin, the papilla in the area of the odontoblasts is capable of producing dentin without further influ-

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ence of the enamel epithelium. However, nothing is known concerning the activity of the ameloblasts when these, together with some already formed enamel, are transplanted and thus segregated from the influence of the odontoblasts. The aforementioned authors did not transplant the enamel organ with the first few increments of enamel. It would be interesting to know whether or not the ameloblasts are capable of independent function *after* amelogenesis begins.

It is generally acknowledged that ameloblasts form the enamel matrix in which calcium salts are deposited. For a long time the source of the latter was considered to be the enamel organ. Doubt has long been cast on this concept by investigators (8, 9, 10) who maintained that calcification of the enamel was effected by both the enamel organ and the dental pulp (papilla). Enamel calcification appears to proceed in two stages, suggesting the terms "primary and secondary calcification" (10). Chase (11) intimated this sequence by showing the marked difference in acid solubility of the enamel matrix. Weinmann, Wessinger and Reed (12) added to this concept by showing that enamel in the matrix state contains approximately 35% mineral matter. In the mature state, the percentage of mineral salts is raised to 95%. The source of calcification of enamel has been a subject of speculation. As previously mentioned, Beust and Bodecker (8, 9) maintained that calcification of the enamel originated from both enamel organ and pulp. Chase (11) attributed the entire calcification of enamel to the ameloblast. Diamond and Weinmann (13) suggested that the complete quota of calcium salts originating from the enamel organ is present in the matrix state of the enamel in colloidal form and then crystallizes in the maturation stage.

PAPILLECTOMY

In a previous publication (14) the roentgenologic result of experimental papillectomy on the teeth of cats was reported. It showed that slight resorption of the peripheral tooth tissues occurred. Intraosseous eruption proceeded at a retarded rate. Furthermore, some evidence of growth was observed. The papillary area contained tissues of varying densities.

The technic of papillectomy (14, 15) overcomes some of the objections to transplantation experiments and casts further light on the phenomena associated with enamel formation and calcification. It minimizes trauma as a factor, permits separation of tooth germ elements and allows further growth and development of tooth components without change of environment. Other phenomena associated with tooth development, such as eruption, or tissue replacement in the papillary area may also be studied. Investigations of the effect of amelectomy, papillectomy and variations of both are now in progress.

The purpose of this communication is to report histological evidence of the effect of experimental papillectomy on various tooth elements.

OPERATIVE TECHNIC AND PROCEDURES

Under ether anesthesia, an incision was made on one side of the mandible through the soft tissues overlying the outer border of the jaw of young cats.

The tissues were retracted and a window was cut through the cortical bone with a small surgical bur. The intact dental papilla, including the entire odontoblastic layer, was then removed from the developing permanent canine tooth through its apical region, and the wound sutured (14). The developing canine tooth of the opposite side of the mandible served as a control in each case. Post-operative recovery was uneventful.

The histological findings of these experiments will be described in the following phases.

I. Effect of papillectomy on the enamel organ and forming enamel.
II. Repair of the papillary area.
III. Influence of papillectomy on eruption.

OBSERVATIONS

I. *Effect of Papillectomy on the Enamel Organ and Forming Enamel.* *Fig. 1* is a section of the dental papilla after removal from the tooth. It has the typical appearance of almost avascular embryonic connective tissue. The odontoblastic layer (O) was removed with the papilla.

Fig. 2 is a photomicrograph of the lower canine of a kitten $3\frac{1}{2}$ months after papillectomy. The enamel epithelium in the incisal area has become transformed into squamous epithelium (S Ep). In one area cyst formation (C) has occurred. In other areas the reduced enamel epithelium appears normal or is absent. Proceeding further apically, the enamel organ and some of the enamel matrix appear to have been resorbed (ER). The enamel epithelium has disappeared completely in these areas.

It may be recalled that the degree of calcification of the enamel is evaluated by its acid resistant quality (13). Enamel in the matrix state resists decalcification and possesses an affinity for hematoxylin. At this time the matrix contains approximately 35% of mineral matter (12). Calcification increases the inorganic salts to approximately 95% and in this state the matrix is acid-soluble and the structure is lost in decalcification. A further, often misleading observation, is the degree of shrinkage in the matrix state, which occurs during the histologic preparation. The present knowledge of the pattern of secondary calcification facilitates recognition of artifacts caused by shrinkage (13).

Fig. 3 illustrates the pattern of secondary calcification and also the difference between mature or acid soluble enamel (ASE), and artifacts caused by shrinkage (S). Calcification begins at the cuspal tips and progresses in horizontal layers from the occlusal surface to the cervix, which is the last area to undergo secondary calcification (13). The specimens (*figs. 2* and *4*) described in this paper present evidence of marked shrinkage, due probably to faulty fixation of the tissues. The large spaces (*fig. 2*, S) do not indicate that a part of the enamel has undergone secondary calcification. The enamel in area "M" is at the same stage of development as that at "M′," as both are formed during the same period. Consequently, the fact that there is little enamel matrix at "M" is due to shrinkage and dislocation because the area "M" shows an abundance of matrix. Hence we conclude that the enamel is in the immature or matrix state.

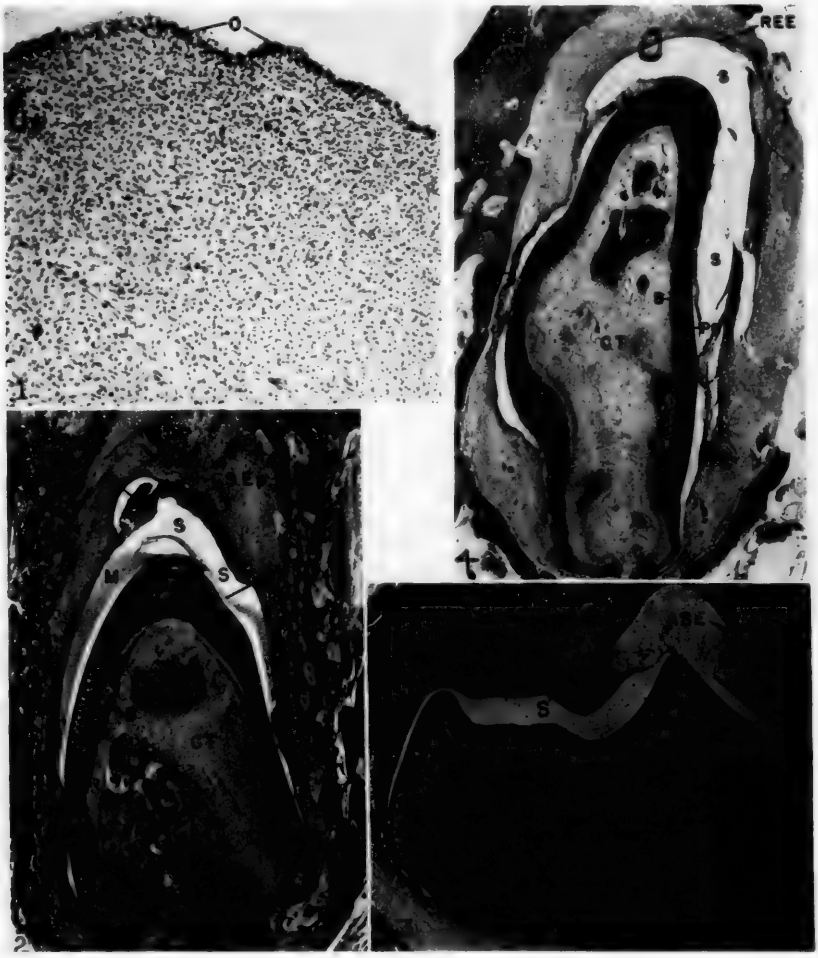

Fig. 1. Section of papilla after removal from tooth germ. Odontoblasts (O). 175 × *orig. mag.*

Fig. 2. Section of lower canine tooth of kitten 3½ months after papillectomy. No odontoblasts are present. Enamel epithelium transformed into squamous epithelium (S Ep); cyst formation (C); apparent resorption of enamel matrix (ER); artifact caused by shrinkage (S); enamel matrix (M-M′); connective tissue (CT); bony process (BP); bone-like tissue resembling cementum (B); resorption (R); predentin (PD). 17 × orig. mag.

Fig. 3. Illustration from Diamond and Weinmann (13) demonstrating pattern of calcification. Mature or acid-soluble enamel (ASE); enamel matrix (M); artifact caused by shrinkage (S). 10 × orig. mag.

Fig. 4. Section of lower canine tooth of cat 7 months after papillectomy. No odontoblasts are present. Reduced enamel epithelium (REE); enamel matrix (M); apparent resorption of enamel matrix (ER); artifact caused by shrinkage (S); connective tissue (CT); bony process (BP); bony-like tissue resembling cementum (B); spike formation of bone-like tissue (SF); predentin (PD). 17 × orig. mag.

Fig. 4 is a photomicrograph of a papillectomized canine which was permitted to remain in situ for 7 months before necropsy. The reduced enamel epithelium is less evident than in *fig. 2*. In areas where it is missing, a type of resorption seems to have occurred. The enamel is still in an immature state, i.e., secondary calcification has not occurred. In areas where the reduced enamel epithelium is absent, the enamel matrix appears to be covered by another structure, separating it from the surrounding connective tissue.

Fig. 5. Section of normally developing control tooth for specimen shown in *fig. 2*. Area occupied by enamel (E). 15 × orig. mag.

Fig. 6. Section of normally developing control tooth for specimen shown in *fig. 4*. Area occupied by enamel (E). 12 × orig. mag.

Fig. 5 is the control tooth for the specimen shown in *fig. 2*. This normally developing, partially erupted canine shows that the enamel had been dissolved in the process of decalcification. Similarly, *fig. 6*, the control for the specimen shown in *fig. 4*, a normally developing, completely erupted canine, shows the same result. It should be noted that in the case of each of the controls, with the pulps intact, the enamel was completely dissolved, an indication that secondary calcification had been completed.

Examination of the enamel under higher magnification reveals further interesting observations. *Fig. 7* shows a normal area of the enamel matrix and reduced enamel epithelium in a papillectomized tooth. Here, even though the enamel organ did not undergo any degenerative changes, no secondary calcification of the enamel occurred, leaving it in the matrix state. As mentioned previously, secondary calcification was complete in the control tooth.

In *figs. 2* and *4* a narrow pink-stained tissue was seen covering the enamel in isolated areas. Higher magnification (*fig. 8*) demonstrates the change which has occurred. It is known that enamel forms from within outward. An alteration in tinctorial reaction of this tissue may be observed. Under normal conditions the enamel matrix stains blue with hematoxylin. In these specimens, however, the transverse striations of the individual rods extend into a pink-staining periphery. The reduced enamel epithelium is no longer present and the surrounding connective tissue is in contact with the amorphous extension of the enamel rods. *Fig. 9* demonstrates this change at a later stage. The enamel rod sheaths continue peripherally in segments defined by the transverse striations. The stain values change even more markedly than in the initial stage (*fig. 8*). The last segments of the enamel rods fuse into a homogeneous stratum, and the rods lose their identity. *Fig. 10* demonstrates a still later stage in which the hyalin-like covering of the enamel may be identified. The outermost layer can be recognized as bone, due to the presence of embedded osteocytes.

II. *Repair of the Papillary Area.* The areas previously occupied by the papilla were filled with various mesodermic tissues. *Fig. 2* shows the repaired area $3\frac{1}{2}$ months after the operation, occupied mainly by adult connective tissue (CT) and blood vessels. A bony process (BP), extending from the apical alveolar bone, occupies the central portion, which shows evidence of active bone resorption and bone formation. The calcium-poor inner surface of the dentin (predentin) is covered with a bone-like tissue (B) similar to osteo-cementum. The connective tissue serves to attach the bony lining of the dentin (B) to the bony process (BP). Area "R" is a perforation caused by resorption progressing from without inward. With the exception of "R," no resorption progressing from within outward is evident in the dentin.

A similar result may be observed in the 7 month specimen (*fig. 4*). The area from which the papilla was removed is occupied by adult connective tissue (CT). The bony process (BP) does not appear to be continuous with the alveolar process. The deposition of a bony cementum-like tissue (B) on the predentin is more advanced. In some areas it resembles spike formation of cementum (SF). No resorption of the inner surface of dentin is evident.

In both specimens (*figs. 2* and *4*) no odontoblasts are present. The fibroblasts differentiated into cementoblasts or as osteoblasts. The formation of additional calcified tissue may be described as cementum or bone. *Fig. 11* demonstrates the similarity between the repair of papillectomy and a normal paradontium. The structures noted are closely similar to cementum, periodontal membrane and alveolar bone. The difference is that these tissues

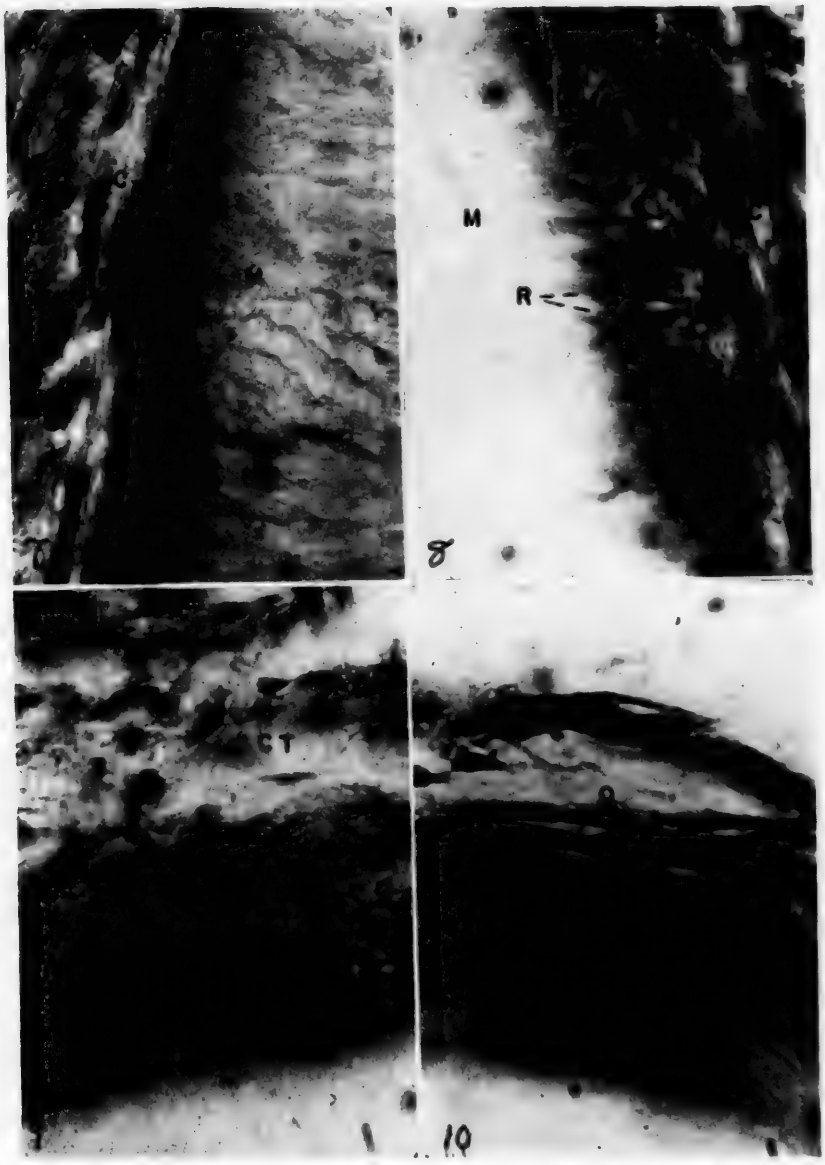

Fig. 7. Normal area of enamel matrix and reduced enamel epithelium. Enamel matrix (M); cuticle (C); reduced enamel epithelium (REE). 900 × orig. mag.

Fig. 8. High magnification of "resorbed" enamel matrix (M) from *fig. 4*. Stained rod sheaths (R) in close apposition to connective tissue (CT). 900 × orig. mag.

Fig. 9. Another enamel matrix area (M) from *fig. 4*. Enamel rod sheaths (RS) with marked transverse striations are being replaced by hyaline pink staining structure (S); connective tissue (CT). 900 × orig. mag.

Fig. 10. Still more advanced stage than that shown in *fig. 9*. Pink staining structure is recognizable as bone by presence of osteocytes (O); enamel rods (R); matrix (M); osteocytes imbedded in outermost layers. 900 × orig. mag.

formed within the pulp cavity following papillectomy. The bonelike tissue (B) deposited on the predentin (PD) closely resembles cementum. Lamellae may be observed at "L." Imbedded cells resembling osteocytes or cementocytes are present. A thin uncalcified zone similar to precementum also may be seen. The connective tissue (CT) between the pseudo-cementum (B) and bony process (BP) has assumed the character of a true periodontal membrane.

Fig. 11. High magnification of papillary area from *fig. 4*. Note resemblance to normal paradontium. Dentin (D); predentin (PD); bone-like tissue resembling cementum (B); lamellae (L); connective tissue resembling normal periodontal membrane (CT); bony process resembling alveolar bone (BP). 250 × orig. mag.

Bundles of connective tissue fibers with fibroblasts may be observed attaching cementum-like tissue (B) to the bony process (BP) found in the pulp chamber. The cementum-like tissue (B) appears to be undergoing continuous formation whereas resorption and formation of the bony process is evident.

In both specimens (*figs. 2* and *4*) small islands of epithelium are distributed throughout the connective tissue. These have the appearance of squamous epithelium and resemble the debris of Mallassez. The origin of these epithelial cells is unknown. They are probably part of Hertwig's epithelial sheath, dis-

lodged during operative procedure. Unlike the epithelium found in the periodontal membrane, these islands are eventually encapsulated with collagenous fibers. In later stages the epithelial cells undergo pyknosis and the tissue is replaced by bone.

Fig. 12 shows an island of epithelial cells within the adult connective tissue in the papillary area. *Fig. 13* demonstrates a later stage of encapsulation of another group of epithelial cells. *Fig. 14* demonstrates a still further change. The collagenous fibers have increased in density. The tissue still lacks the true appearance of bone and the epithelium is beginning to lose its characteristics. A still later stage may be observed in *fig. 15*. The epithelial cells bear only a slight resemblance to their normal appearance, due to degenerative changes. The presence of enclosed osteocytes identifies the surrounding tissue as bone.

III. *Influence of Papillectomy on Eruption.* The roentgenographic report on papillectomy (14) described the retarded rate of eruption of the papillectomized teeth. Histological evidence verifies the roentgenologic findings.

Resorption of the bony crypt in the incisal portion and bone building in the apical portion of papillectomized teeth indicates that eruption has continued (*figs. 2* and *4*). Under higher magnification, (*fig. 16*) the apical area of the $3\frac{1}{2}$ month specimen may be observed. Bone formation (BF) in the direction of eruption is evident. Resorption of the bony process (BP) in the opposite direction defines the movement of the bony process towards the oral cavity (arrow). *Fig. 17* is a photomicrograph of the incisal area of the bony crypt. Resorption (R) of the bone in this area indicates eruption.

In the 7 month specimen the eruption is more evident. In the apical portion (*fig. 18*) the bone deposition is manifested by a layer of osteoblasts on the bone facing the apex. Slight evidence of resorption on the opposite surface of the trabecula may be observed. *Fig. 18* shows the incisal portion of the bony crypt of the same specimen. Resorption of this surface is evident. Resorption and bone formation of the bony process within the papillary area also denote eruption (*fig. 19*). Resorption facing the apical area and bone formation toward the oral cavity describe the eruptive process.

DISCUSSION

I. *Effect of Papillectomy on the Enamel Organ and Forming Enamel.* This investigation verifies the reports of other investigators (4, 5, 6, 7) inasmuch as it is shown that the dental papilla is essential to amelogenesis. In other experiments (1, 2, 3, 4, 5) transplantation of the enamel organ demonstrated that this tissue was incapable of function without the dental papilla after enamel formation had begun. The present findings agree with the previous authors and elaborate still further on the relation between the enamel organ and dental papilla.

Following papillectomy, the enamel organ is incapable of retaining its identity. Most of it becomes transformed into squamous epithelium and undergoes cystic degeneration similar to observations made on transplants.

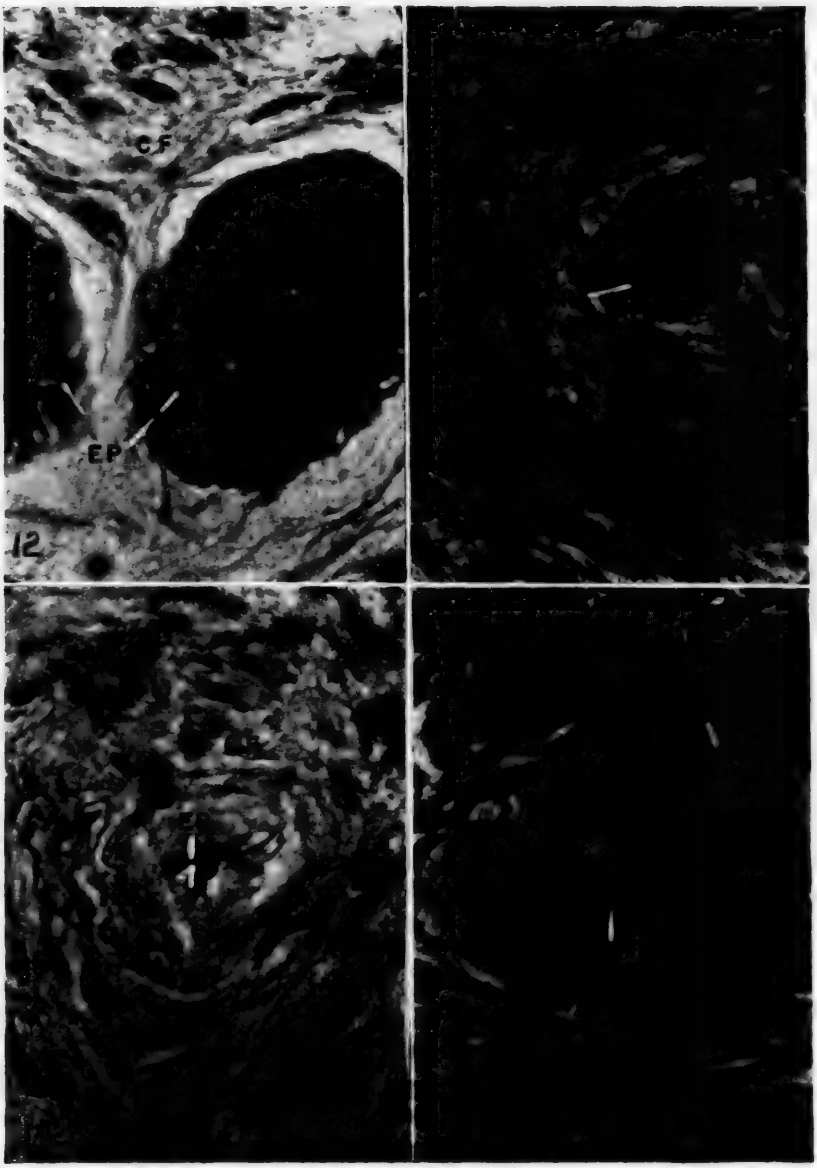

Fig. 12. High magnification of papillary area from *fig. 2.* Cluster of epithelial cells (EP) surrounded by collagenous fibers (CF). 900 × orig. mag.

Fig. 13. High magnification of papillary area from *fig. 2.* Later stage than that shown in *fig. 12.* Note increase in collagenous fibers surrounding epithelium. Cluster of epithelial cells (EP); collagenous fibers (CF). 900 × orig. mag.

Fig. 14. High magnification of papillary area from *fig. 2.* Later stage than that shown in *fig. 13.* Note beginning degeneration of epithelial cells (EP); collagenous fibers (CF). 900 × orig. mag.

Fig. 15. High magnification of papillary area from *fig. 2.* Later stage than that shown in *fig. 14.* Note advanced degeneration of epithelial cells (EP). Osteocytes (Oc) in calcified structure designate it at bone. 900 × orig. mag.

The hypothesis that secondary calcification is dependent upon the enamel organ is disproved by the evidence presented in this report. In areas where degeneration of the enamel organ occurred, there is no evidence of secondary calcification (*fig. 2*). This would suggest that the enamel organ is the causative factor of such calcification. However, even in areas where the reduced enamel epithelium is normal (*fig. 7*), the enamel matrix did not undergo secondary calci-

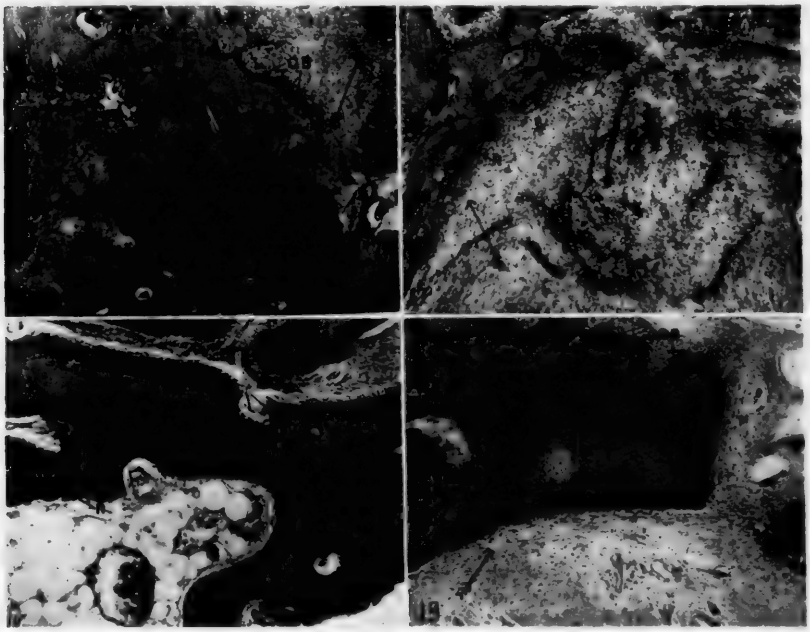

Fig. 16. High magnification of apical region from *fig. 2*. Arrow indicates direction of eruption. Note bone formation in direction of eruption indicated by presence of osteoblasts (OB); osteoclasts (OC). 150 × orig. mag.

Fig. 17. High magnification of incisal region from *fig. 2*. Arrow indicates direction of eruption. Resorption (R). 150 × orig. mag.

Fig. 18. High magnification of apical region from *fig. 4*. Arrow indicates direction of eruption. Bone formation indicated by presence of osteoblasts (OB); resorption (R) on opposite side of trabecula (T). 150 × orig. mag.

Fig. 19. High magnification of incisal area region from *fig. 4*. Arrow indicates direction of eruption. Bone formation designated by osteoblasts (OB) and lamellae (L) in trabecula (T) demonstrate direction of eruption. 150 × orig. mag.

fication. In the control teeth (*figs. 5* and *6*) secondary calcification was completed. The difference in the papillectomized tooth is that the dental papilla and the odontoblasts are missing. This finding further casts doubt upon the hypothesis that the inorganic salts are present in colloidal form in the enamel matrix and that they crystallize in the maturation stage. The evidence shows that calcification beyond the matrix state does not occur in the absence of the papilla and odontoblasts.

Histological observations (8, 9, 10) suggested the hypothesis that the dental pulp was active in enamel calcification. Evidence of vital staining methods showed that the dental lymph passed from the dental pulp, through the dentin into the enamel (16). Further evidence that dental lymph may be a calcifying agent was presented by one of the authors (17). Saunders, Nuckolls, and Frisbie (18) discussed the function of the interprismatic spaces and suggested that they "provide a temporary channel for fluid exchange in the desiccation and consolidation of the matrix."

Inasmuch as dental lymph is the only tissue fluid which has been traced from the pulp into the enamel, it may be assumed that the dental lymph plays an important rôle in the continued calcification of the enamel, as demonstrated by experimental papillectomy.

The replacement of enamel by bone, as indicated in *figs. 8, 9* and *10* is inexplainable at this time. Cahn (19) reported the influence of epithelium on heterotopic bone formation. These findings suggest that a fine line of differentiation may not be drawn between the potentialities of different germ layers.

Resorption of the surface of the enamel suggests that the vitality of the enamel matrix is lost after removal of the odontoblasts. Gottlieb (20) stated that the reduced enamel epithelium prevents resorption of the enamel prior to eruption. The findings in papillectomy suggest that the papilla and odontoblasts are associated with preservation of the reduced enamel epithelium. It is possible that the enamel matrix derives its vitality from the odontoblast (16). Loss of this cell layer, therefore, may cause loss of vitality and subsequent resorption of enamel.

The terms primary and secondary calcification are misleading. Examination of enamel in the matrix state demonstrates a degree of calcification (13). It is generally assumed that the enamel matrix is formed as a partially calcified tissue. Bodecker (21) demonstrated the presence of an organic matrix in fully calcified enamel. Gies and Rosebury (22) identified this substance as a protein resembling keratin. Nuckolls, Saunders and Frisbie (18) suggest that the enamel organ lays down a keratin matrix (an epithelial product) which is subsequently calcified. The findings following papillectomy postulate a continuous process of calcification. The enamel epithelium forms the organic matrix, in which inorganic salts are deposited. The papilla and odontoblastic layer seem to be the significant factors in the calcification process. The low degree of calcification found in the experimented teeth occurred while the odontoblasts were present. Upon their removal calcification was arrested. The activity of the odontoblasts, measured by the diffusion of dental lymph suggests that calcification of enamel is a continuous process, most active in young teeth and diminishing with age.

One final observation is presented which also suggests that the enamel is calcified by odontoblastic activity. Diamond and Weinman (13) contributed extensively to the comprehension of enamel calcification. They showed that the process begins at the summit of the cusp (*figs. 21* and *3*). This area is nearest to the enamel organ and farthest from the dental pulp. In order to

ascertain the source of the elements necessary for calcification, an examination of the pattern of bone and dentin growth is necessary. It is an acknowledged fact that calcification always begins at an area most remote from the calcifying organ. Hence the work of Diamond and Weinmann (13) gives added evidence

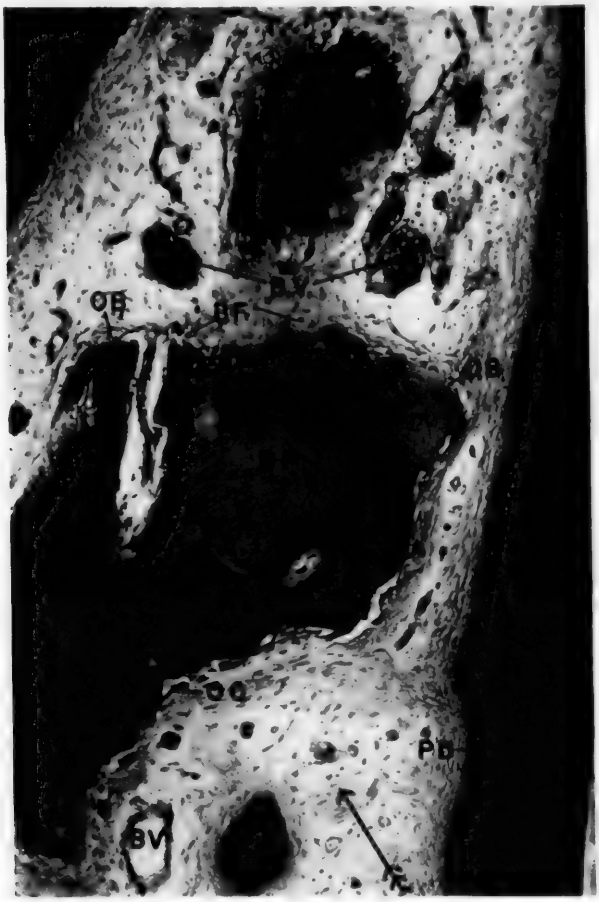

Fig. 20. High magnification of papillary area from *fig. 4*. Bony process (BP) within papillary area shows evidence of resorption and formation (BF). Osteoclasts (OC), osteoblasts (OB). Note prolific blood supply. Blood vessels (BV); predentin (PD). 150 × orig. mag.

that the calcification process originates mainly from the interior, i.e., the odontoblasts.

A study of enamel calcification indicates that it is a slow and continuous process. The group of odontoblasts (*fig. 21*, 1′) are the first formed cells; they have affected the zone of matrix "1" longest. Hence, it is comprehensible that calcification begins at this area. The odontoblasts in zones 2 to 10 are

also in communication with the enamel matrix, but no secondary calcification has occurred because the process is not as yet sufficiently advanced. The reason is that the calcification phenomenon is slow. About 3 months elapse before acid-insoluble enamel becomes acid soluble.

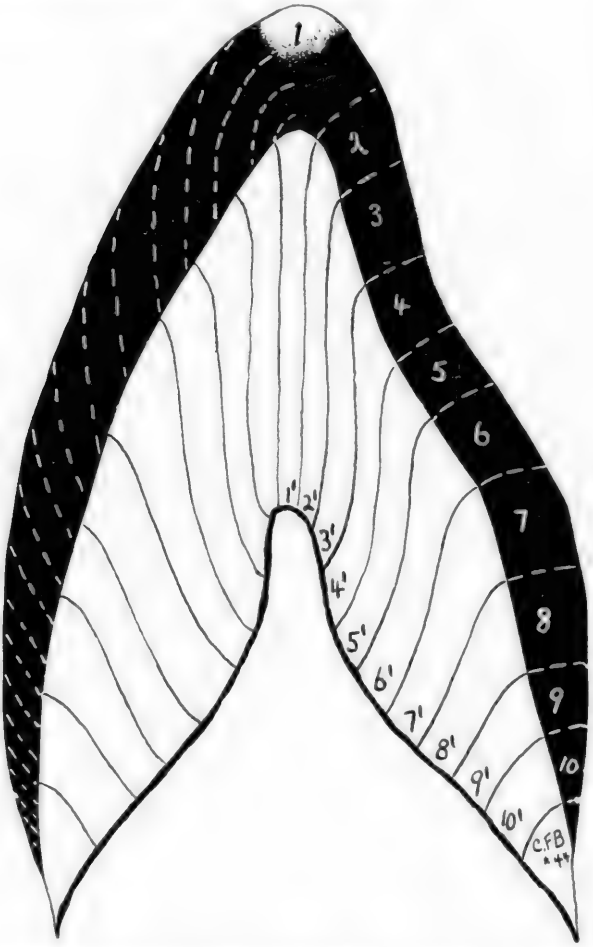

Fig. 21. Diagram adapted from Diamond and Weinmann demonstrating secondary calcification of enamel. Matrix formation proceeds according to the incremental lines (striae of Retzius) on left of diagram. Secondary calcification occurs approximately at right angles to these, noted on right.

II. *Repair of the Papillary Area.* The formation of a cementum-like lining of the dentin contributes to Gottlieb's (20) theory of resorption and formation of calcified tissue. He suggests that the "quality" or degree of calcification determines whether the surrounding connective tissue will cause resorption or

formation of calcified tissue. This theory also implies that a tissue which is resistant to resorption will stimulate the formation of bone or cementum. The resistance of predentin to physiologic resorption is well known (23). It is interesting to note that no resorption of the predentin surface occurred after papillectomy. Resorption occurred in one area (R, *fig. 2*) from without—inward. The formation of a bone-like tissue on the calcium-free predentin suggests that an uncalcified surface encourages the formation of calcified tissue.

This may also explain the formation of cementum on the surface of the root. The first formed peripheral root dentin, containing the granular layer of Tomes, includes many non-calcified areas. Comparison of the peripheral dentin and predentin may be made in *fig. 20*; both areas have a similar tinctorial value. Thewlis (24–25) demonstrated the lack of calcification of peripheral dentin which, in the root portion, is the well known granular layer of Tomes.

Histological characteristics of the calcified tissues depend upon differentiation of fibroblasts. In the absence of the influence of the ameloblasts, the fibroblasts do not differentiate into odontoblasts. The calcified tissue formed in the pulp chamber resembles bone or cementum. Thus the differentiated fibroblasts designate the type of calcified tissue formed and its microscopic anatomy. Fibroblasts differentiated into odontoblasts form dentin, and when differentiated into cementoblasts or osteoblasts, cementum or bone forms. Glasstone (7) and Fish (26) made a similar observation of a bone-like tissue forming on the inner surface of dentin which they called "scar tissue." No odontoblasts were present in the area. Wolbach and Howe (27) found a similar result following degeneration of the odontoblasts in avitaminosis C.

Fig. 11 shows an interesting arrangement of connective tissue fibers within the papillary area. These bear close resemblance to an adult periodontal membrane under stress. The orientation and number of principal connective tissue fibers in the normal periodontal membrane are believed to be formed as a result of masticatory stress. There is no doubt that this observation is true, for it is known that non-functioning teeth show a great reduction of principal periodontal fibers which lose their orientation (28). During the growth of the pseudoperiodontal membrane, however, no stress other than eruption has been brought to bear on the teeth making this a rather puzzling observation.

Previous investigations have established the inability of ameloblasts to cause differentiation of odontoblasts following amelogenesis (4, 5, 6, 7). The degeneration of the epithelium following papillectomy may prevent the enamel organ from stimulating the formation of odontoblasts. Experiments in ameloectomy now in progress may contribute to the solution of this problem.

Another interesting phenomenon observed is the encapsulation of epithelial cells by bone. It appears as though the epithelial cells influence the formation of collagenous fibers from the surrounding connective tissue. Calcification of these fibers into bone is followed by degeneration of the epithelial cells.

III. *Influence of Papillectomy on Eruption.* The attempt to explain the mechanism of tooth eruption has resulted in a large number of diverse theories. Such mechanical factors as the elongation of the root of the tooth, pressure of

the dentin and constriction of the dental pulp, pressure exerted by the muscles of the cheek and tongue, resorption of the alveolar crest, and various tissue tension theories are among the current hypotheses. Massler and Schour (29) have challenged the validity of the prevailing theories and have suggested another factor, the vascularity of the tissue which surrounds the tooth. In a more recent publication, Sicher (30) advanced the explanation that "it is the growth of the pulp in a restricted proliferative zone at the apical end of the tooth above the inflected epithelial sheath and the osteogenic tissue preceding bone apposition which combine to supply the 'force' of eruption."

The existing theories do not explain the intraosseous eruption following papillectomy. In repair of the papillectomized area, the connective tissue shows a greatly increased vascularity over that of the normally developing control tooth (*fig. 20*). If, therefore, vascularity were the sole influencing factor in eruption, we should expect an increased rate of eruption which did not occur.

SUMMARY AND CONCLUSIONS

1. Following papillectomy, histodifferentiation of fibroblasts into odontoblasts does not occur.

2. Degeneration of the enamel organ and cyst formation occur after removal of the dental papilla and odontoblasts.

3. The evidence suggests that enamel calcification is a continuous process dependent upon the presence of the papilla and the odontoblasts, stressing the conclusion that the dental papilla and the odontoblasts are *essential* to enamel calcification.

4. The pattern of calcification of enamel is evidence that the calcification occurred from *within, not from without.*

5. Dental lymph explains the only known histologic connection between enamel and odontoblasts.

6. Fibroblasts in the papillary area differentiate into osteoblasts.

7. The calcium-poor surface of dentin and cementum is conducive to apposition and resists resorption.

8. Intraosseous eruption of the papilla-less tooth occurs at a slow rate as compared with the normally developing control tooth.

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STUDIES ON THE pH OF SALIVA[1]

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There is now general agreement that saliva is more often slightly acid than basic; also, there is a growing opinion that considerable fluctuations in the pH of saliva make studies on the problem of questionable value. In a previous report Eisenbrandt (1) showed that repeated tests of saliva upon a few individuals gave an average pH of 6.72 which was comparable to data of investigators who reported on single tests made upon many individuals. Brawley (2) reported 6.75 as the average pH of saliva, Grossman and Brickman (3) 6.7 and Swerdlove (4) 6.69. Others have made similar observations. In arriving at average values considerable dissension existed as to the methods and procedures of determining the hydrogen ion concentration of saliva.

Eisenbrandt (1) also reported that the saliva of subjects was significantly variable in the pH, and that a significant cycle occurred during the day; the lowest pH was at 9 A.M. and the highest at 5 P.M. These results tended to agree with those of Grossman and Brickman (5). Broderick (6) observed that investigators had found a great range in the pH of saliva which caused some of them to doubt the importance of salivary pH. Cognizance should be made of how such doubts arose from experiments in which single tests were made upon many subjects. Broderick firmly believed that there are definite causes for these variations and expressed a desire for an eventual explanation.

The purpose of this paper is to report the data which accumulated during 1 year on the salivary pH of a few subjects. The data were studied to obtain a mean for the group, to study monthly variations and bi-hourly variations of the group and to study individual means and variations at monthly and bi-hourly intervals. Also, the paper is intended to demonstrate that the pH variations of saliva are not haphazard, but display characteristics for the group and for the individual.

METHODS

The hydrogen ion concentration of saliva was expressed in terms of pH and was determined by utilizing an electrometer and a glass electrode. The procedure was the same as reported in (1). Approximately 5 ml. of unstimulated saliva were collected in the mouth and then deposited into a sample cup; the reading was taken within 1 minute. Environmental influence was not great during this short interval of time, therefore, the exclusion of air from the sample was unnecessary.[2]

[1] Received for publication April 24, 1944. Revised by author August 14, 1944.

[2] Repeated tests on the effect of air exposure upon the pH of saliva confirmed this statement.

Readings were taken repeatedly upon the same subjects for 1 year at bihourly periods (9 and 11 A.M. and 1, 3, and 5 P.M.) for 1 week each month. The initials of the subjects served for identification. H joined the W.A.C. and was replaced by R; M entered the experiment after the second month; the remaining 4 subjects were available during the entire 12 months. No consideration was given to differences in sex; the group was composed of 2 women and 5 men.

The statistical method was the same as applied in (1). Because extensive tables were required to present completely the statistics, condensation was deemed advisable. In designating significance the following key was employed: ** denoted significance, i.e., the ratio resulting from the calculations was at least equal to the 1% point; * denoted probable significance, i.e., the ratio was between the 5% point and the 1% point; — denoted no significance, i.e., the ratio was less than the 5% point. Many investigators hold that the 5% point is sufficient to indicate significance. No distinction was made between the terms "mean" and "average."

EXPERIMENTAL DATA

The experiment extended over a period of 1 year and consisted of 1552 tests on the pH of saliva of 7 subjects. The average pH for this number was 6.64 with a standard deviation of ± 0.254 and a standard error of the mean of ∓ 0.006. The pH range of single samples was from 5.28 to 7.48.

Monthly Variations of the Group

In addition to the mean pH of saliva, the data were considered for monthly variations of the group. An analysis of variance clearly demonstrated that the mean pH for the group was significantly variable during the 12 months of testing. See Table I and *fig. 1* for the details. The pH was highest during October and November and lowest in May; another low point was in January. The range of monthly means was from 6.55 to 6.72. The data suggested a biseasonal fluctuation in the pH of saliva because the elevations in the pH were in the fall and early spring. Attention is called to the last few months where a general increase in pH approached the range of the fall months. Although the experiment terminated in September, 1943, additional tests were made on 4 of the 7 original subjects during November. The data indicated a close parallelism with the beginning of the original experiment in September, 1942.

In addition to the analysis of variance, the standard error of the mean for each month was calculated and given in Table I. Five monthly means for salivary pH were significantly different from the annual mean, 2 were probably significant and 5 were not significant. Those for October and November were definitely greater than the mean, those for February and March were probably greater and for January, May and July, they were significantly less than the mean pH for the year. Therefore, the pH of saliva for the group varied markedly during the year; the changes seemingly fell into seasonal patterns. About

half of the monthly means deviated definitely from the annual mean of pH 6.64.[3]

Monthly Variations of Individuals

A comparison of the annual variations of the salivary pH among the 7 subjects left no doubt of the marked variability (see Table II). The lowest pH

TABLE 1

Analysis of monthly variations in the pH of saliva

	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	TOTAL
No. readings	135	117	83	115	120	140	118	119	145	149	148	145	1552
Mean pH	6.72	6.72	6.61	6.56	6.65	6.65	6.66	6.53	6.62	6.58	6.66	6.67	6.64
Std. error of mean	∓0.018	∓0.025	∓0.030	∓0.023	∓0.020	∓0.020	∓0.028	∓0.022	∓0.022	∓0.017	∓0.021	∓0.018	∓0.006
Total mean ratio	4.33**	3.14**	1.00	3.45**	1.98*	2.04*	0.70	4.03**	0.92	3.46**	0.94	1.63	

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	1551	100.0179	
Between monthly group means	11	5.1085	0.464409
Readings within months	1540	94.9094	0.061629

F = 0.464409/0.061629 = 7.54 ..... significant
1% Significance = 2.24

** = Significantly different from 6.64

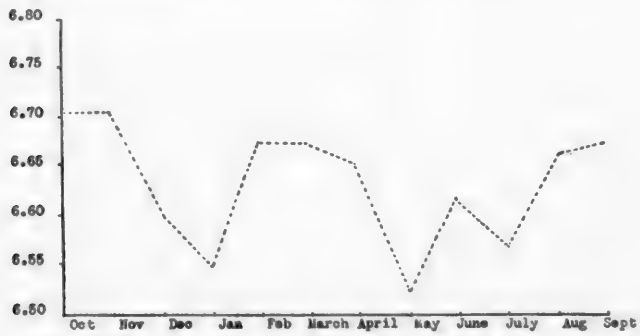

Fig. 1. Monthly averages of the pH of saliva for the group.

mean was for P, who averaged 6.52 and ranged from pH 5.28 to 7.26. C had 6.54 for a mean pH with a range from 5.74 to 7.40. The highest mean pH

[3] pH is the logarithm of the reciprocal of the hydrogen ion concentration. Although averaging pH is a common procedure, it is theoretically erroneous. Each pH reading should be translated into $[H^+]$, calculations made and then the final figures converted back to pH. However, since the pH of saliva rarely fluctuated beyond the pH 6–7 range, it was deemed desirable to resort to the common practice of averaging pH without converting to the $[H^+]$.

was 6.77 for both H (range 6.29 to 7.42) and M (range 6.19 to 7.48). Each subject had at least 100 tests made on his saliva; moreover, 5 of the subjects averaged approximately 265 tests. Such data demonstrated that the individuals varied from one another in pH characteristics of saliva, and also suggested that each person had an average pH which characterized him. *Fig. 2* illustrates the annual mean of each subject. The group mean is included for comparison.

Fig. 3 shows how each subject varied from month to month. Five of the 7 subjects were significantly variable from month to month. C was an exception

TABLE II

Analysis of variance on the pH of saliva of 7 subjects

	H.	S.	C.	M.	P.	E.	R.	GROUP
No. readings	140	268	258	231	280	275	100	1552
Mean pH	6.77	6.70	6.54	6.77	6.52	6.66	6.59	6.64

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	1551	100.0179	
Between subjects	6	13.8260	2.304333
Between readings of each subject	1545	86.1919	0.055788

F = 2.304333/0.055788 = 41.31 ..... significant
1% Significance = 2.80

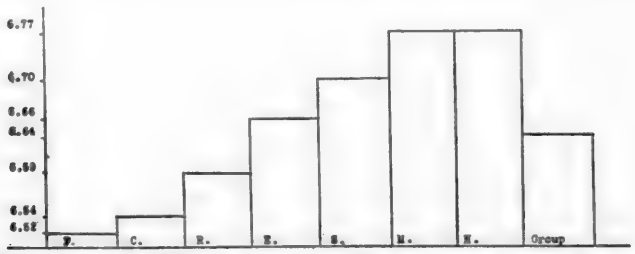

Fig. 2. The annual mean pH of saliva for each subject and for the group.

in that the pH of his saliva was one of the lowest in the group and had no appreciable variations throughout the year. His range of monthly means was from pH 6.39 in December to 6.66 in both October and February. The other subject whose pattern did not vary on a monthly basis was R, who joined the staff in June and only 4 months (100 tests) of her record were available. During this period her monthly means varied only from pH 6.56 to 6.62. Note should be made, however (*fig. 3*), that R's monthly means were similar to the last 4 months of the other subjects so far as the general pattern was concerned. Likewise, the pattern of H resembled others at the beginning of the study. The

5 subjects who varied markedly demonstrated clearly that the pH of saliva was not a fixed entity, but changed from month to month. Of these P had the greatest variability, ranging in monthly means from 6.29 in January to 6.72 in October. His yearly mean pH of 6.52 was the lowest of any individual in the

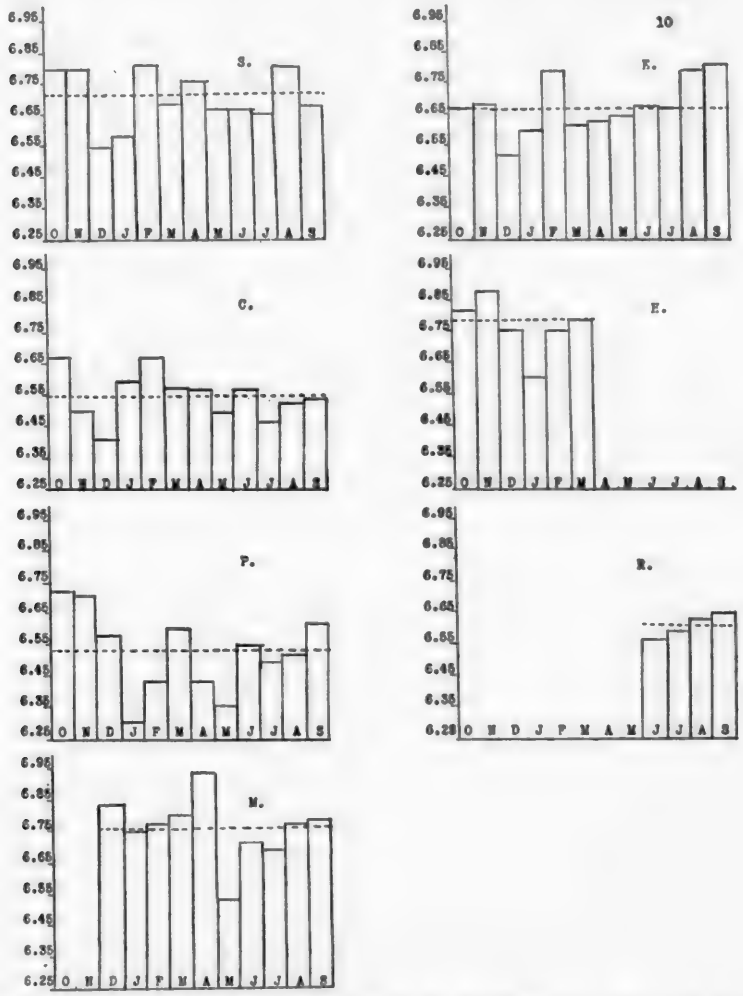

Fig. 3. The monthly means for each subject; the dotted line in each graph denotes the subject's average.

experiment. In contrast to P was M who had practically as much variability of means from month to month as did P, but who had a relatively high mean pH of saliva. The pH range of the monthly means of M's saliva was between pH 6.54 in May and 6.94 in April.

Another set of data are illustrated in *fig. 3* in which subjects are compared each month. An analysis of variance of each subject's salivary pH for each month showed striking results. The subjects were dissimilar in average pH each month because the variability was significant for 10 months and probably significant for 2 months, October and June. The greatest variations were in January, February and April. Therefore, during each month the subjects were considered significantly variable from one another which in turn signified that saliva had pH values characteristic for each individual. This is in agreement with the annual data for the subjects (Table II).

In summary of the individual studies, the pH of the subjects' salivas varied significantly from one another for the year and for each month. Also, 5 subjects were variable from month to month during the year, and 2 subjects were not significantly variable. Therefore, the characteristics of salivary pH were individualistic, the nature of which altered during the year on a monthly basis.

TABLE III

Bi-hourly variations in the pH of saliva for one year

	9 A.M	11 A.M.	1 P.M.	3 P.M.	5 P.M.	TOTAL
No. readings	310	316	312	310	304	1552
Mean pH	6.55	6.67	6.64	6.65	6.71	6.64

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	1551	100.0179	
Between bi-hourly periods	4	4.0325	1.008125
Between readings in each period	1547	95.9854	0.062046

F = 1.008125/0.062046 = 16.25 ..... significant
1% Significance = 3.32

Bi-hourly Variations of the Group

Another problem considered was the pH of saliva at different times of the day, i.e., bi-hourly intervals from 9 A.M. through 5 P.M. The general results for the year confirmed the preliminary data reported in (1). The low point for the group was at 9 A.M. with a pH mean of 6.55 followed by a rise at 11 A.M. to 6.67. The high point was at 5 P.M. when the pH of saliva averaged 6.71. Reference to Table III shows that over 300 readings were made for each bi-hourly period, totaling 1552 pH determinations. The graph is illustrated in *fig. 4*. An analysis of variance for the bi-hourly periods demonstrated that the data were highly significant. It is to be noted that, in addition to the general upturn during the day, the graph flattened during the early afternoon and passed through the annual mean of 6.64 at 1 P.M.

The bi-hourly data for the year were divided into each time unit and considered for the group, for the comparison of subjects at a particular period and for each subject during the day. The monthly data for the group were also

studied for each period and graphed in *fig. 5*. The pH of saliva at each bi-hourly period was significantly variable over the year except at the 3 P.M. period which was probably significant. Only by repeated tests for any one

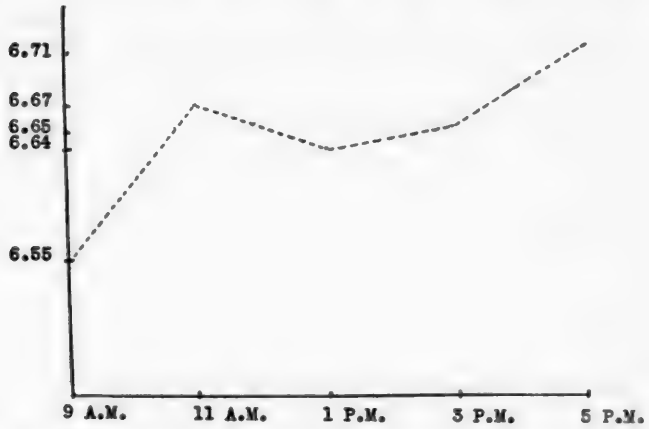

Fig. 4. The mean pH of saliva for the group at bi-hourly intervals.

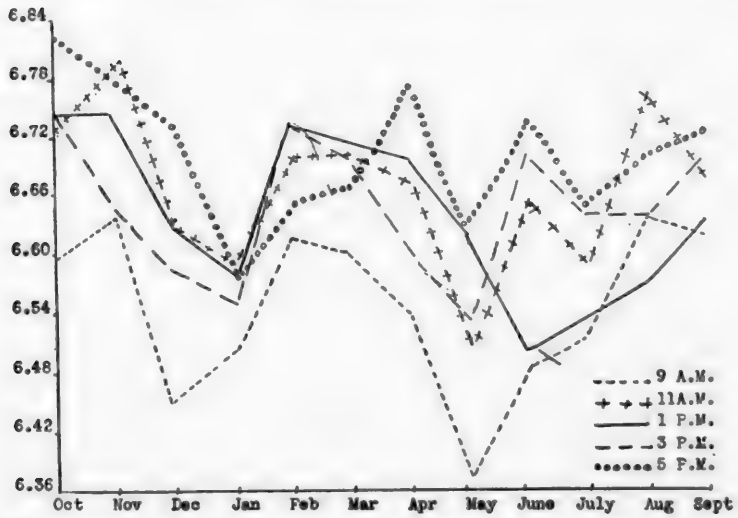

Fig. 5. How the pH of saliva varies at each bi-hourly period during one year, and how the five periods vary from one another at each month.

period were definite pattern characteristics evident (also true for subjects). This partially explains the prevailing opinion that single readings have little or no meaning.

Although the saliva at the 9 A.M. period had an average pH (6.55) definitely

less than at other periods, there was a significant range of the 9 A.M. monthly means from 6.37 in May to 6.64 in August and November. The 11 o'clock averages for each month were significantly variable, and although definitely higher, closely paralleled those of 9 o'clock. Also, the monthly curve for 1 o'clock varied significantly during the year. Whereas the pH of saliva at other periods during June markedly increased, the average at 1 P.M. made a decided drop. Reference to *fig. 5* shows how the monthly average for the 3 o'clock period tended to change less than for other periods, especially was this true during the last 3 months of the experiment. However, the statistical analysis of the 3 P.M. monthly pH means showed the probability of the monthly variability being significant. The remaining period (5 P.M.) was significantly variable during the year; it tended to be less acid than the other periods although it dropped below the 11 A.M., 1 and 3 P.M. periods during February and March.

An analysis of variance of salivary pH at bi-hourly periods for each month was determined. During the early summer months the pH of saliva at the bi-hourly periods was definitely more variable than during the late winter and early spring. The greatest variability was in June and the least in January. The range of means for the bi-hourly periods at each month is illustrated in *fig. 5*. In January the pH of saliva dropped markedly for all periods except 9 A.M., which, although lower than the other periods, tended to increase over the preceding month. There were increases in February for all periods.

In general, the pH of saliva for the group was significantly variable from month to month during the experiment and the degrees of fluctuation between bi-hourly periods remained approximately the same for each month. The pH of saliva was lower at 9 A.M. than at any other time, except in August when the 1 o'clock became lower; also, the saliva was usually higher in pH at 5 P.M. than at other periods. When the bi-hourly variations in the pH of saliva were broken into monthly units, the variations were not as marked except during the summer and in October.

Bi-hourly Variations of Individuals

The next unit considered was a comparison of the salivary pH of subjects at each bi-hourly period, and the variations of each subject's saliva during the day (Table IV). At 9 A.M. the 7 subjects were highly variable (statistically significant). P's saliva had a pH value of 6.39 and C's was practically identical, whereas H's saliva had the high value of 6.76. The 11 o'clock tests were highly significant in variability of subjects. The lowest pH was P's saliva, the highest was H's. The subjects were highly variable (significantly so) at 1 o'clock, but this time C had saliva with the lowest pH (6.48) and M had the highest (6.90). The same high significance of variability among the subjects was evidenced at 3 P.M., P was lowest and H was highest. The final period, 5 P.M., likewise was highly significant in variability. R had the low pH reading of 6.58 and H had 6.80. The high degree of variability was about the same for all periods, except for 5 P.M., which although marked, was decidedly less than the others. The divergence of subjects at each time period is clarified in *fig. 6*.

The second part of Table IV (reading down) deals with the variability of saliva of each subject during the day. Four of the 7 subjects were significantly variable in pH changes of saliva for the 5 bi-hourly periods, 1 was probably significant and 2 were not. The latter were H and R for whom incomplete annual records were available. During the day the salivary pH of S was the most variable, followed in diminishing order by C, M, P, E, R and H (*fig. 6*). Therefore, subjects varied notably from one another in salivary pH at each bi-hourly period, however, in general each subject tended to follow the usual pat-

TABLE IV

The pH of saliva studied over a period of one year showing how subjects varied from one another at specific times of day; also, how each subject varied during bi-hourly intervals

	S.	C.	P.	M.	E.	H.	R.	ANALYSIS OF VARIANCE
(9 A.M.)								F..... 13.61
No. readings....	54	52	53	47	56	28	20	1% sign.... 2.90
Mean pH.....	6.55	6.40	6.39	6.66	6.62	6.76	6.65	Sign..... **
(11 A.M.)								F..... 12.76
No. readings....	54	54	58	46	56	28	20	1% sign.... 2.90
Mean pH.....	6.76	6.58	6.52	6.75	6.72	6.79	6.57	Sign..... **
(1 P.M.)								F..... 13.45
No. readings....	55	49	58	46	56	28	20	1% sign.... 2.90
Mean pH.....	6.65	6.48	6.57	6.90	6.62	6.69	6.58	Sign..... **
(3 P.M.)								F..... 13.42
No. readings....	54	51	56	47	54	28	20	1% sign.... 2.90
Mean pH.....	6.77	6.55	6.50	6.76	6.66	6.78	6.58	Sign..... **
(5 P.M.)								F..... 4.79
No. readings....	51	52	55	45	53	28	20	1% sign.... 2.90
Mean pH.....	6.78	6.68	6.62	6.75	6.71	6.80	6.58	Sign..... **
Analysis of Variance								
F.....	10.07	9.00	6.26	7.76	3.07	1.27	1.49	
1% sign.....	3.41	3.41	3.41	3.41	3.41	3.47	13.57	
Sign.....	**	**	**	**	*	—	—	

tern of the entire group during the day as illustrated in *fig. 4*. Also, the subjects who were in the experiment for the entire year had saliva which altered in pH to a marked extent at the bi-hourly periods during the day.

The final phase of this report was an analysis of the bi-hourly data for each individual from month to month. Most subjects did not vary sufficiently to be significant. Of the 35 calculations, 27 were not significant, 5 were probably significant and only 3 were significant. P was significantly variable at 9 A.M. during the year and M was at both 9 A.M. and 5 P.M. No one varied significantly at 1 P.M. Reminder is made that saliva at this period had an annual

mean pH of 6.64 which was the same as the mean for the total number of tests. In general, each subject failed to vary appreciably from month to month at a particular time of day. The greatest variations seemed most likely at 5 o'clock, and then at 9; the least variations occurred at 1 o'clock. This added importance and strength to the bi-hourly variations during the day, making them definite entities.

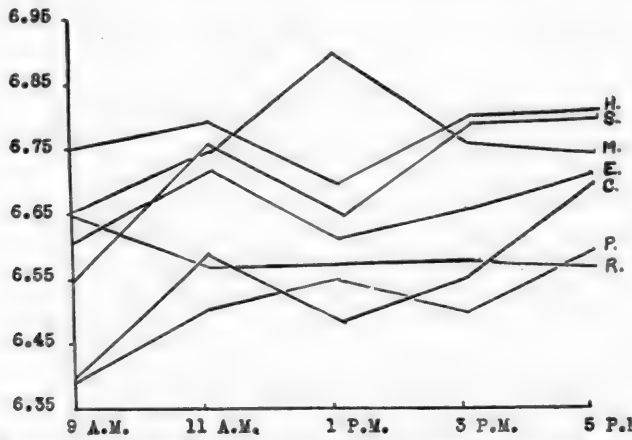

Fig. 6. Variations in the pH of saliva at bi-hourly intervals for each subject, also, between subjects for each time interval. Repeated tests were made for one year.

DISCUSSION

The relatively low mean pH of saliva of 6.64 obtained in this experiment is interesting in that it is lower than most investigators report. Swerdlove (4) approached this figure with a mean of 6.69. The present report shows that repeated tests for 1 year provided a more accurate mean than tests for a brief time as in the preliminary report of the writer (1) where 6.72 was the mean pH for the same group when tested in the months of October and November. The lower mean for the year is accounted for by the marked decline in the pH of saliva during the winter and again during the late spring. Since the earlier report was for a period of only 2 months, the low points which were possibly due to seasons were missed. The need for long range studies on the pH of saliva is emphasized because of the significant fluctuations during the year. In addition, these fluctuations help explain the haphazard data and conflicting conclusions frequently reported in the literature in which experiments dealt with single readings irrespective of the time of year or the time of day. It is highly desirable to state these periods when making reports on the pH of saliva. The possibility that the electrometer was influenced by seasonal changes was eliminated because it always checked properly with the standards. Also, that the instrument was not at fault was further proved by the fact that occasionally a subject's saliva increased in pH while that of the others decreased.

The standardization of technics in determining salivary pH is absolutely

required before investigators can produce comparable results. Becks (7) stressed this point and commented that environmental and seasonal variations as well as general metabolism may influence the characteristics of saliva. The present report adds weight to Becks' supposition. Krasnow (8) also stressed the need for standardizing technics and factors affecting pH. Reference to the literature well portrays the variety of technics. Saliva may be stimulated or nonstimulated; it may be fresh or several hours old; it may or it may not be exposed to the air; it may be incubated or refrigerated before testing. The above exemplifies the need for controlling the actual collection of the saliva and the time elapsing before testing. Usually various colorimetric methods have been employed to determine the pH of saliva. The electronic method with the glass electrode is preferable because of its accuracy, but unfortunately the method has been little used. On the basis of this experiment it became evident that individuals differ from one another as to the pH of saliva, and that they have individualistic daily, monthly, and annual cycles. The resulting graphs resemble one another in character but vary in degree. Failure to consider these cycles may be a partial answer to the vacillations on the pH studies of saliva and their possible relationships to the biological phases of dentistry.

The variations during the day have several interesting points. The lowest pH of saliva was found at 9 A.M. and this varied markedly with the subject. There is a suggestion of the relationship with body metabolism, because the pH of saliva increases during the day especially between 9 and 11 A.M. when metabolic activities usually become greater. Associated is the observation by Starr (9) that the pH of saliva increases during excitement. The present experiment indicates that saliva has a pH at 1 o'clock (one half-hour after lunch) equivalent to the annual mean. This is indicative of the preferable time to perform single tests. The relationship to the pH of saliva to other properties of saliva and possible correlation to dental problems will be reported later.

SUMMARY

(1) The mean pH of saliva of 7 subjects was 6.64 $\mp$0.006, which was based upon 1552 electrometric tests made with the glass electrode at regular bi-hourly intervals for an entire year. The statistical analysis of variance aided in interpreting the results.

(2) The pH of saliva for the group varied markedly during the year and indicated seasonal fluctuations.

(3) The subjects were significantly variable for each month and for the year, although each one tended to follow the general trend of the group.

(4) Each subject displayed individualistic salivary pH characteristics.

(5) The pH of saliva of the group was lowest at 9 A.M., was always greater at 11 A.M., passed through the annual mean at 1 P.M. and was highest at 5 P.M.

(6) The pH of saliva for the group was significantly variable from month to month at each bi-hourly period.

(7) The pH of saliva was compared at the 5 bi-hourly periods for each month. The variability was significant only in May, June, July, and October.

(8) The subjects varied markedly (in salivary pH) from one another at each bi-hourly period.

(9) Each subject tested throughout the year had saliva which varied significantly in pH during the day at bi-hourly intervals.

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MUTATIONS OF BACTERIA UNDER DIETARY INFLUENCE[1]

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The geographic distribution of the degenerative diseases of civilization parallels in an exact and striking manner the world wide distribution of those diseases of mankind and animals which are known or are alleged to be caused by streptococci. In general, the incidence of the diseases known to be of streptococcal etiology and the degenerative diseases vary indirectly with the mean annual rainfall, temperature and ultraviolet radiation, i.e., they become less frequent as the equator is approached. However, in the arctic regions where these factors are not operative, these diseases are practically non-existent, a phenomenon which makes it apparent that diet is a great factor governing the distribution of streptococcal and degenerative diseases.

Primitive people, regardless of their geographic distribution, have in common two factors of great fundamental importance: First, a natural unmanipulated diet and; second, a non-pathogenic basal body flora. The basal body flora of isolated communities living in the tropics, in the temperate zone and in the arctic regions is remarkably constant and it is characterized primarily by a lack of pathogenic streptococci, pneumococci, diphtheria bacilli and other pathogenic organisms. *Streptococcus pyogenes* is essentially an organism of civilization and industrial overcrowding. The significant difference, bacteriologically, between primitive people, domesticated and wild animals and breast-fed babies on the one hand, and civilized human adults on the other, is the constant presence of pathogenic streptococci in the basal flora of the latter group and the absence of pathogenic streptococci in the former group. Upon this latter phenomenon may, perhaps, rest the explanation for the rarity of certain diseases in primitive people in contrast to their excessive incidence in civilized communities. The modern diet is the factor in civilization which is chiefly responsible for the differences bacteriologically in the basal flora of primitive and civilized human beings.

It is a matter of common observation throughout the world that when primitive diets are supplemented with sugar and flour, dental caries and the other diseases of civilization make their appearance. Concomitantly with the ingestion of flour and sucrose, pathogenic streptococci, pneumococci and other organisms foreign to the primitive body flora make their appearance. The basal oral flora of dogs, cats, cows, calves, mice and breast-fed babies is similar and is characterized by its simplicity, its lack of pathogenic organisms and of organisms

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which rapidly produce acids, and the absence of streptococci in the mucoid state. *Streptococcus salivarius* is the predominant streptococcus (*figs. 1* and *2*).

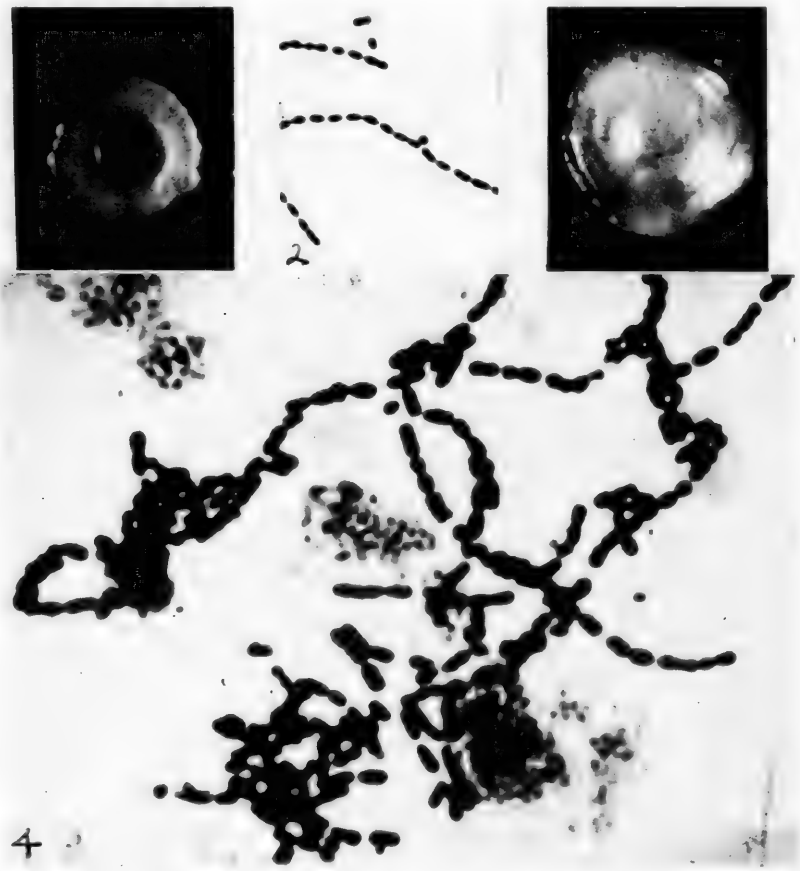

Fig. 1 (*above left*). Colonial formation of *Streptococcus salivarius* as derived from breast-fed baby. Medium is nutrient blood agar. × 20.

Fig. 2. *S. salivarius* derived from breast-fed baby and grown in a soy bean infusion. × 1,000.

Fig. 3 (*above right*). Colonial formation of *Streptococcus odontolyticus* derived from the saliva of caries susceptible individual.

Fig. 4. Pleomorphism of *S. odontolyticus* when grown in cabbage infusion. About × 1400.

Within a short time after a baby is taken from the breast and placed on a diet containing sucrose and cereal, changes occur in the oral flora in a direction leading toward that which is found in adult civilized human beings. The flora becomes more complex and more acidogenic, pathogens occur with frequency, and many of the streptococci exist in a transitional or frankly mucoid state

(*figs. 3* and *4*). At first, organisms make their appearance which resembles both *S. salivarius* and *S. pneumoniae*, while later, more pronounced changes occur. By streaking the saliva of a sucrose, cereal-fed baby on blood agar plates containing 1% sucrose, it has been found that the majority of the streptococci produce mucoid colonies.

The characteristic and striking difference in the flora of the saliva from breast-fed babies and the majority of civilized adults, when streaked on sucrose-containing medium and incubated 48 hours, is the absence of the mucoid streptococcal colony from the plate of the former, and its presense on the plate of the latter. It is this mucoid form of *S. salivarius* which has been named the odontolytic variant, or more specifically the *Streptococcus odontolyticus*.

The oral streptococci exhibit an indifferent behavior in the R and S phases, in contrast to the mucoid phase where they exhibit an ability to become adjusted to a wide variety of environments and will grow in practically all carbohydrate infusions, rapidly producing large amounts of acid. Streptococci in the M phase exhibit a tendency to develop "mutational" forms with altered morphology, staining reaction, biologic characteristics and pathogenicity. The influence of diet on bacterial evolution for the science of immunology is significant for diet is probably the greatest single factor in causing "spontaneous" changes in microbic virulence and in governing the rise and fall of epidemics.

The changes which occur in the oral flora of the baby after it has been placed on the modern diet can be reproduced in vitro by the use of media containing sucrose and cereals, and, to a less extent, other foods. Sugars other than sucrose are only slightly effective in causing this conversion. *S. salivarius* is the predominant and characteristic organism of mammalian saliva. When first cultured from the mouths of mammals or breast-fed babies and plated on sucrose containing medium, streptococcus salivarius grows in small, flat, non-mucoid colonies and is non-pathogenic. Sucrose, by providing an unfavorable initial environment, accelerates bacterial changes and favors the development of "mutational" forms. When *S. salivarius* is grown in the presence of sucrose and cereal, its growth is first depressed and later accelerated, acid production is enhanced, and mutational forms may make their appearance. When the mutational forms of *S. salivarius* induced by the sucrose, are grown in an infusion of cereals, crude or refined, the evolutionary tendency is maintained and continued in the direction of further variation. It is occasionally possible by cultural methods to convert *S. salivarius* into mutational forms which when plated on nutrient blood agar plates containing 1% sucrose, produce mucoid colonies indistinguishable from those constantly found in the saliva of individuals on the modern diet.

In the test tube, the crude cereals, as well as the refined, favor the development of the mucoid state, but in the saliva, it is only the refined products which have much significance in the maintenance of this mucoid state. In contrast to the whole grain products, the refined products do not sustain antibiosis and therefore permit the selective growth of the acidogenic streptococci.

When the mutational forms induced by sucrose are grown in the presence

of potato, but more particularly potato-sucrose, the evolutionary tendency, in contrast to that occurring in cereals, is not sustained, the organisms revert to the primitive level they occupied on primary isolation. Although soy beans, and perhaps other foods consumed by human beings, may sustain the evolutionary step induced by the sucrose, it is probable that only the refined cereals are of much clinical significance in this connection. From these observations it would appear that cereals, even in the refined form, could be ingested without great harm from a bacteriological standpoint *provided the individual was on a sucrose-free diet*. It further seems probable that in the absence of certain foods, particularly cereals, sucrose in limited amounts could be ingested with safety either alone or in conjunction with other carbohydrates.

Once the "shift" to the acidogenic state has taken place in the formerly primitive flora and the oral streptococci have become converted from a smooth vegetative status to the rapidly growing mucoid or evolutionary phase, then practically all carbohydrates consumed by man can be converted into acid with great rapidity. Thus, after the condition of caries hypersusceptibility, as demonstrated by the presence of many organisms in the mucoid phase, has been established, *all* carbohydrates consumed by man, perhaps even protein, contribute to the carious process.

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HISTOPATHOLOGY OF ENAMEL CARIES[1]

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LITERATURE

Miller (1) was the first to investigate carious enamel microscopically. It appeared to him that the interprismatic substance was dissolved, bacteria forcing their way between the loosened prisms. The loosening of the prisms he considered caused by acid, the action of the microorganisms on the enamel being indirect through the acid which they produce. Williams (2) described the beginning of the carious process as a dissolution of the interrod substance, this material being less resistant to the acid of decay. This substance is not the sheath for the sheath, as well as Nasmyth membrane, is more resistant to acid than the prisms. G. V. Black (3) described the process in a similar way. Caries of the enamel, he believed, was caused by acid formed by microorganisms, which lie upon the outside of the enamel and never enter the tissue until the enamel rods are loosened and fall out. C. F. Bodecker (4) stated that the first action of the acid of decay on the enamel is to attack the cementing substance by robbing it of inorganic salts. The bacteria tunnel into the core of the rod, the sheaths and the cementing substance appearing to be more resistant to acid. Appelbaum (5) showed that the Retzius (incremental) lines become more visible in incipient caries. The calcium salts disappear by acid action and the organic framework shows up more clearly.

Beust (6) was the first to call attention to the fact that carious enamel resists acid action and therefore caries cannot consist purely of acid action. Gottlieb (7) described undermining enamel caries, explaining it by the known fact that the deeper layers of the enamel are less calcified than the superficial ones.

OBSERVATIONS

Carious enamel is more resistant to acid than intact enamel. In routinely made histological serial sections through human jaws pieces of carious enamel are often found persisting while surrounding healthy enamel has been dissolved. It has been pointed out that that is apparently due to enriched protein content of the enamel by invading of microorganisms. The following illustrations are made from such specimens, which have resisted the nitric acid used in the histological technique.

Fig. 1 shows such a piece of carious enamel from a decalcified section. At (c) there is the entrance to an undermining carious cavity in the enamel. The deeper parts of the enamel at (d) and below (b) are necrotic. At (e) prisms are

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seen to be isolated and dislocated although the straight parallel prisms at the surface can still be seen. The area (f) below the depression (a), caused by necro-

Fig. 1. Area of carious enamel from decalcified section, resisting acid action. (a)—tooth surface, (b)—undermining cavity, (c)—entrance to the cavity, (d)—necrotic enamel, (e)—dislocated prisms, (f)—area shown at higher magnification in *fig. 2*.

Fig. 2. (a)—enamel surface, (b)—thickened prism sheaths and their transverse connections (empty framework), (c)—prism sheaths black, prisms dark, resisting acid, (d)—no prism sheaths between prisms.

Fig. 3. Area of carious enamel from decalcified section. (a)—enamel surface with necrotic layer, the lines between the prisms empty, (b)—dislocated prisms with some material between.

sis, is shown in *fig. 2* at higher magnification. At (b) a ladder like, dark arrangement is found. That is thickened organic framework, such as can be observed

in enamel with straight, parallel arrangement of the prisms. The spaces between the framework which appear to be empty correspond to the calcified parts of the prisms, which have been dissolved. Between (b) and (c) transverse striations are also apparent but the places between become dark, indicating some remnants of the prisms themselves. At (c) the dark parallel longitudinal lines are quite black and the spaces between are not light (empty) but dark. The black lines are apparently the prism sheaths and the dark strips between the prisms. The black

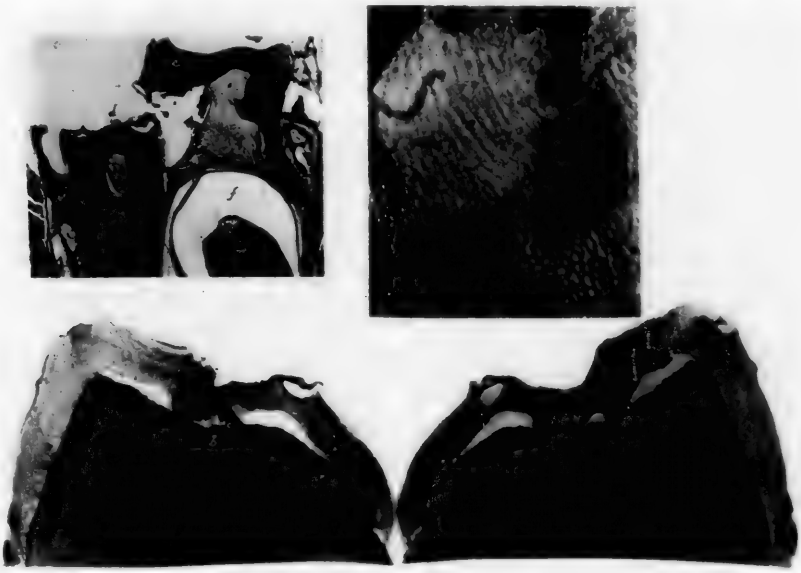

Fig. 4. Decalcified section. (a)—first premolar with only occlusal surface erupted, (b)—persisting enamel matrix, (c)—second deciduous molar, (d)—caries on occlusal surface of premolar. (e)—unerupted second premolar, (f)—mature enamel dissolved, (g)—collapsed organic rests of enamel.

Fig. 5. Fissure caries of tooth (a) of *fig. 4*. (a)—undermining cavity, (c)—coagulated tissue fluid, (b)—undermining dentin caries.

Fig. 5a. Neighboring section of *fig. 5*. (a)—entrance to cavity, (b)—not quite matured enamel matrix having resisted the nitric acid of histologic technique.

Fig. 6. Higher magnification of section of series *figs. 4, 5,* and *5a*. Difference degrees of caries. (a)—intact matrix with prism differentiation. (b)—different pieces of carious sheaths arranged in arcades, the dark areas entirely affected.

stain here as compared with the dark stain at (b) seems to indicate that the carious process has progressed more. The prisms between persisted and were not dissolved by acid which suggest that their protein content was fortified, apparently by invading microorganisms. Towards (d) the black prism sheaths disappear and only the isolated prisms remain. In *fig. 1* it can be observed that these prisms finally end in the necrotic area (d). *Fig. 3* is of a piece of carious enamel from another decalcified section. The dark parallel prisms appear similar to

those in *figs. 1* and *2*. The prisms are dislocated at (b) and some amorphous material fills the place between. The place usually occupied by the prism sheaths is empty as at (d) in *fig. 2*.

Figs. 1, *2* and *3* are illustrations from carious teeth of adults. *Fig. 4* illustrates an erupting first premolar (a), the second deciduous molar (c) and the germ of the second premolar (e). The enamel of this germ was dissolved by acid, indicating its maturity. Only remnants of the organic matter of that enamel can be seen at (g). Some of the enamel of the erupting, older first premolar resisted acid action (b). Corresponding poor calcification is apparently responsible for the fact that extensive caries started when the occlusal surface erupted into the mouth, although the lateral walls were still in connection with the epithelium. The black places on the occlusal surface are carious areas. *Fig. 5* shows a higher magnification of the crown of one section. The dentin is covered by a persistent enamel matrix. In the middle of the occlusal surface there is a carious area. A neighboring section (*fig. 5a*) shows the entrance. Here we see the dentin (b), apparently shrunken and the created vacuum (c) filled with coagulated tissue fluid. That can only be identified as such after having experience in more instructive specimens. The cavity has apparently developed later, (a) being a part of it. Only the last stage of enamel development failed to occur. The stage of prism differentiation had taken place. In an enamel piece from another section of the same tooth (*fig. 6*) at (a) a piece of intact enamel shows the darker prism sheaths and the lighter prisms. The developing caries first affected the less calcified prism sheaths. The black arcades (b) resemble the black lines at (c) of *fig. 2*, prism sheaths affected by caries. In other places there are areas, as those on the right, which are dark like the prisms in *fig. 2*. In the left upper corner a homogeneously black area of necrosis, like (d) in *fig. 1* is seen.

DISCUSSION

It is not possible to study minute histology or histopathology of tooth structures in ground sections alone. Their thickness does not allow examination with highest power. On the other hand decalcified sections must undergo treatment with different chemicals, which may cause important alterations. For study of pathological changes in tooth structures, like caries, both should be used. Ground sections permit observations on unaltered tissue and decalcified sections show details under higher magnification.

In *fig. 2* all details of the different stages of enamel caries can be seen. The area (b) appears to represent the earliest stage of the carious process. The appearance of transverse striation of the prisms in connection with caries is ordinarily considered the result of acid action. As soon as a part of the calcium salts is dissolved the organic framework becomes clearer visible, but this observation is possible only in the superficial layers of thick enamel where the prisms run straight and parallel. In the deeper layers, which are apparently a product of free crystallization, such regular transverse striation can not be observed. Even in ground sections it is apparent that the lines of transverse striation in connection with caries are far thicker than those found in experimental acid action. In the trans-

verse striation of the decalcified section this ladderlike framework is definitely thicker than it would be if it were the freed framework of the enamel. We are not able to make any other suggestion, than that the organic framework has been enriched and thickened by invasion of microorganisms, which represents the carious process.

The question of the place of origin of the organic matter which is necessary for expansion of the prism sheaths when the calcified parts are intact is difficult to answer. This problem was discussed in connection with the thickening of the lamella in beginning caries. An entirely satisfactory explanation cannot be offered. It is possible that the thickening in the ground section is merely an accentuation by increased content. When the specimen is decalcified and the rigid walls removed the compressed content expands.

The spaces between these thickened lines represent the more calcified prisms, which apparently were not affected. Correspondingly they were dissolved by the acid used in the histological technique. Between (b) and (c) the transverse lines are not clearly visible, but the longitudinal lines appear black and darker at (c) than at (b). This might indicate that the prism sheaths are more affected here. The spaces between, which are apparently the prisms, appear as gray strips which have resisted acid action. We suggest as explanation that the disintegration of the prism sheaths has progressed further and that the invasion of the prisms by microorganisms made them resistant to acid. A further advanced stage seems to be present at (d). The black lines of the prism sheath have entirely disappeared, probably by digestion. The prisms are still present, but isolated and dislocated. This stage is seen clearly in *fig. 3*. The material to be seen in one place between the dislocated prisms is probably necrotic. Finally the prisms themselves become necrotic too, as can be seen at several places in *fig. 1* (d). This is the stage of cavity formation in which the disintegration of the tooth tissues has reached the ultimate stage. The stage of advanced disintegration of the prism sheaths is also to be seen in *fig. 6* where some parts of the prism sheath are black (b) as in area (c) of *fig. 2*.

The fact that the deeper parts of the enamel (*fig. 1*) show the more advanced stage of necrosis while the superficial parts of the enamel resist longer, has already been discussed as being due to the higher calcification of the superficial enamel. The tooth in *fig. 4* appears to be only partly matured and already there is an indication of diffuse undermining enamel caries. It is easy to understand that the affected dentin (b of *fig. 5*) is more extensive than the affected enamel. Compared with half mature enamel, dentin is more easily invaded.

The carious process has been seen to progress primarily along the organic roads of the prism sheaths. The discussion of the lamellae is omitted here. It has been proposed that the organic prism sheaths cement the more calcified prisms to each other. In fluorosed enamel transparent strips are found where the prism sheaths are apparently calcified too. This might be the reason why such enamel crumbles easily and, at the same time, the prism sheaths cannot be used as invasion roads, being obstructed by calcification. This may account for greater resistance to enamel caries in teeth formed in high fluorine areas.

SUMMARY

The carious process first invades the prism sheaths while the prisms between appear to be intact. Later the process invades the prisms and increases their resistance to acid. In the advanced stage the prism sheaths become black in the decalcified section and finally disappear entirely, apparently being digested. The prisms remain visible, separated and no longer connected by the prism sheaths. Finally, they become necrotic too. Thus four stages can be differentiated in the progress of enamel caries: First, the organic framework is thickened; Second, the process invades the prisms making them more acid resistant while the prism sheaths turn black; Third, the prism sheaths disappear while the isolated prisms are still present; Fourth, a necrotic mass replaces the enamel structure.

The photomicrographs were made by Chas. Ervin Arnold, Dallas, Texas.

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INHERITANCE OF SUSCEPTIBILITY TO CARIES IN ALBINO RATS (MUS NORVEGICUS)[1,2]

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Tooth decay has long been recognized as perhaps the most common physical affliction of mankind. Although interest in the study of its causes and prevention is of long standing, progress in this phase of dental research has been particularly evident during the past two decades. The combined efforts of dental research specialists, bacteriologists and nutritionists have made possible the view that tooth decay, like most pathological processes, is influenced by a variety of factors. Previously the bacteriologist has been inclined to insist that tooth decay was essentially a bacteriological problem. The nutritionist was even more specific and no less certain that a deficiency of this or that mineral or vitamin was responsible for the trouble. No one will deny that both of these fields are still most important, but to get a more complete picture we must include two additional factors—that of the physical characteristics of the diet and of heredity. The latter is the subject of the present study.

This investigation was undertaken at the suggestion of Steggerda of the Carnegie Institution of Washington. Steggerda and Hill (1) studied the incidence of dental caries among persons of Dutch descent living at Holland, Michigan, Maya Indians, Navajo Indians, and Negroes from Jamaica. Their observations indicated that there might be hereditary racial differences in susceptibility to caries, which prompted Steggerda to suggest our study of the inheritance factor in rats. The experiments with laboratory albino rats were begun in February, 1937.

There were two objectives at the outset: (1) to determine whether there is an inheritance factor in the development of dental caries in albino rats; and (2) if there is such a factor, to discover if possible the number of gene pairs involved and the genetic and physiological effects of each gene. The first of these objectives has now been attained. The data to be presented will prove that susceptibility and resistance to caries in rats are in part due to heredity. Our

[1] The writers wish to express their great appreciation for financial support of this research by the National Research Council and the American Philosophical Society, and particularly for several very generous grants from the William John Gies Fund of the American College of Dentists.

[2] Presented at the 22nd Annual Meeting of the International Association for Dental Research at Chicago, Ill. on March 19, 1944 (*J. D. Res.*, **23**: 205, 1944). Received for publication, June 17, 1944.

purpose is now to identify the number of genes concerned, if this is possible, and the mode of action of each gene.

We now know beyond doubt that inherited differences are caused by definite stable units, called genes, each of which is located at a specific point on a chromosome. The interested reader can secure the essential facts concerning heredity from a number of well-written text-books on genetics [Sinnott and Dunn (2), Snyder (3), etc.].

It is now generally recognized that an organism is the resultant of the activities of its genes and of the environmental factors to which it has been subjected during development. A method is now available, the path coefficient technique of Wright (4), for quantitatively evaluating the relative importance of heredity and environment in specific situations. We find that susceptibility to tooth decay in rats is determined both by the genes and by the nature of the food. Our 7 years of work on this problem have revealed fascinating vistas of research on heredity-environment relations which we have not yet had the opportunity to exploit.

Results secured with rats obviously, by themselves, prove nothing concerning dental caries in man. Neither do medical experiments on the lower mammals demonstrate anything about disease processes in human beings. In both instances additional observations must be made on man himself. Nevertheless, experimentation on animals has proven invaluable in medical research because it indicates what may be, and often is, true for humans.

EXPERIMENTAL DIET

The discovery by Hoppert, Webber, and Canniff (5, 6) of a caries-producing (cariogenic) diet provided an essential tool for this study. They found that the following mixture ensured health, growth, and fertility in albino rats, but that it caused caries, chiefly in the lower molars.

Coarsely ground hulled rice	66%
Whole milk powder	30%
Alfalfa leaf meal	3%
Sodium chloride	1%

Although the initial diet contained corn as the cereal, polished rice was subsequently used because of its greater effectiveness in producing caries, and its greater uniformity in composition. A very interesting fact is that the rice must be ground coarsely if this diet is to be most effective in producing dental caries. By "coarse" is meant that the rice is prepared in a precision grinder set to crush the grain so that about 70% of it will be retained on a 20-mesh screen (400 holes per square inch) when sifted. The grinder used was made by the Hobart Manufacturing Company of Troy, Ohio. G. E. Braunschneider (a former graduate student in Zoology) secured 15 rats derived from the 8th generation of our susceptible line. He fed them from weaning time on the above diet with the rice ground finer, but not to the fineness of flour. These animals developed cavities at a mean age of 87.3 $\pm$ 4.8 days. The mean age at which rats first showed caries in our 9th generation susceptibles was 64.4 $\pm$ .7 days. The difference,

22.9 ± 4.9 days, is statistically significant. Braunschneider likewise fed about 57 rats (derived from the 8th generation of our susceptible line) on the caries producing diet from weaning time, but the rice was ground to the fineness of flour. This treatment was continued from 100 to 150 days. Only 4 of these animals showed dental decay, and in these 4 it was delayed to an average age of 125 days. The 9th generation animals were put on the ration containing the coarse rice at 35 days of age. They developed dental caries, on the average, about 30 days later. Thus only 7 percent of Braunschneider's 57 rats acquired caries on the mixture containing the rice flour, and these 4 animals required about 3 times as many days to do this as the 9th generation susceptibles when eating the diet containing coarse rice. Braunschneider's investigation is as yet incomplete, and none of his results have been published until now.

Because the percentage of coarse rice particles is such a decisive factor, many screening tests of the ground rice have been made. One hundred grams of the freshly ground rice are sifted through 20- and 40-mesh screens. The fractions retained by the 20-mesh, and 40-mesh screens, and the residue that goes through the latter are weighed and recorded. The first test was in January, 1939. During the intervening 60 months, 50 screening tests were recorded, and a number were not recorded. Thus there has been about one test each month. The grinder is adjusted whenever the percentage deviates too much from 70. New burrs have been installed at intervals of about 15 to 18 months. The following summary shows the consistency with which we have maintained the grade of coarseness of the rice.

Per cent retained by the 20-mesh screen	Number of screen tests
60	1
68	7
70	8
71	11
72	19
74	3
76	1
	—
	50

The average of these 50 tests is 70.9%. Nine-tenths of them are within the range 68% to 72%, inclusive. This degree of precision has been adequate to reveal the hereditary differences to be reported, but it seems highly desirable to eliminate altogether such deviations as 74% and 60%, now that we are trying to breed a homozygous resistant (or immune) stock.

PROCEDURE

Every rat, except those cited in Braunschneider's experiments, was placed on the caries-producing diet, containing the coarsely ground rice, when 35 days old. The food mixture with the finely ground rice was in the cage until the animal reached this age, except when the mother nursing her litter in this cage was a resistant individual that had not developed cavities. In such cases the caries-producing food was in the cage until the young were 15 days old for the benefit

of the mother, then the fine rice mixture was substituted until the young were 35 days of age. Weaning begins not far from 15 days after birth, so the coarse rice diet could have had little or no effect before that age.

The lower molar teeth were examined every 14 days, and the location, approximate size, and date of observation of every cavity were entered in a permanent record book. A diagram of the lower molars, made with a rubber stamp, was used for making these records. The living rat was grasped quickly and firmly with the right hand by the loose skin on the dorsal side of the neck, drawing the neck skin taut so that the animal could not wriggle sidewise and bite the holder, the body was straightened by grasping both hind legs with the left hand, the mouth was opened with a nasal speculum under a bright lamp, and the cavities in the lower molars were noted and recorded. Nearly all the examinations were made by the same person, so that whatever bias may have existed in identifying cavities did not affect comparisons between the susceptible and resistant lines.[3]

A few simple rules were necessary for defining the date on which a cavity appeared. One observation was sufficient if the hole was so large that its existence was beyond doubt. A positive identification of a small cavity was confirmed by observations at one or more subsequent examination periods. In such cases dental caries was considered to have appeared at the date of the first positive observation. When it was impossible to make a definite diagnosis, a "?" sign was entered on the tooth map. The next observation often showed that there was no cavity at the site. Sometimes several successive fortnightly examinations left doubt about the suspected cavity, though finally culminating in one or more positive diagnoses. In such cases the cavity was considered to have appeared at the date of the "?" immediately before the first positive determination. The "caries time" was the number of days between the date the rat was put on the caries diet (at 35 days of age) and the date the first cavity was considered to have appeared. As previously indicated, a resistant mother sometimes nursed a litter before she had developed tooth cavities. The coarse rice mixture was then kept in her cage until the young were 15 days old, when the fine rice mixture was substituted. Such a mother would be returned to the caries-producing diet on removal from her litter, when the young were perhaps 25 to 30 days old. The number of days this female ate the fine rice mixture was not included in her "caries time".

The cages were made of galvanized sheet iron, with a removable litter tray, and closed against drafts on all sides except the top and front, which were covered with $\frac{1}{4}$-inch galvanized iron mesh. The cages were 12 inches high, 14 inches wide, and 20 inches long. Water from the College deep well was supplied constantly from drip bottles. The room temperature was maintained automatically at about 77°, except in hot summer weather, when it was considerably higher.

[3] We are greatly indebted to Mr. Leo J. Klever, Superintendent of the Animal House of the Department of Zoology, for consistently faithful care of the rat stocks, and particularly for his skill at the times of examination in handling the individuals, regardless of their size or ferocity.

As a rule, no more than 4 or 5 animals were kept in one cage, sometimes only 1 or 2. Our original plan was to discard all litters of less than 5 rats, and to reduce the larger litters to 6 soon after birth. The aim was to keep the litter size practically constant. However, it became advisable to abandon this standard at the 4th generation of the resistant line and the 6th generation of susceptibles, because sterility, still births, early deaths of young, and small litters threatened to terminate these lines, or at least to make the continuance of the experiment difficult. Perhaps the close inbreeding was partially responsible for these weaknesses.

BREEDING METHODS

The first objective of the investigation was to determine whether there is an inheritance factor in susceptibility and resistance to dental caries in the albino rat. We therefore undertook to build a caries susceptible line, and a caries resistant line, using phenotypic selection, brother x sister inbreeding, and progeny testing of breeders as genetic techniques to effect this purpose. It was desirable to start with a considerable number of animals from a variety of sources to increase the chance of securing at the outset as large an assortment as possible of genes for susceptibility and resistance. The following rats comprised our first generation: 18 from the colony of the Psychology Department at Michigan State College, 17 from the Nutrition Laboratory of the Home Economics Division, and 84 from the rat laboratory of the Chemistry Department.

Selection was fairly stringent at the outset, for only 16 animals (13.4%) chosen as breeders produced young that were themselves mated. These were: susceptibles, 2 males (1 from the Psychology laboratory, 1 from the Chemistry laboratory), 5 females (2 from Psychology, 2 from Chemistry, and 1 from Home Economics); resistants, 3 males (all from the Chemistry colony), and 6 females (1 from Psychology, and 5 from Chemistry).

The object in selecting and intensively inbreeding by brother x sister matings was not only to build a susceptible and a resistant line, and thereby to demonstrate the inheritance factor, but also to create homozygous susceptible and resistant types which would be crossed, so that genetic segregation could be observed among the grandchildren from such matings, supplying information by which we might determine the number of pairs of genes that are involved. Wright (7) has computed that inbreeding by brother x sister crosses reduces heterozygosity on the average by about 19% of that in the preceeding generation. Thus homozygosity should be approached fairly rapidly in the early generations.

Such inbreeding procedure is known to have several effects: (1) to increase homozygosity; (2) to subdivide the stock into homogeneous lines, each of which is likely to differ from the others; (3) to reveal recessive mutations; and (4) to cause decline in vigor, at least in some of the families, as a consequence of the appearance of recessive traits that adversely affect fertility, resistance to disease, growth, etc. We have maintained only those lines of descent which were useful in building resistance or susceptibility. A few variations that may be hereditary have appeared, but there has been no time to study them. We have encountered

a considerable amount of sterility among breeders and early deaths among young. Inbreeding was probably not the only cause of infant mortality. Early in 1939 an epidemic of what was diagnosed as a streptococcus infection killed many young produced by the 2nd and particularly the 3rd generations of resistant parents.

An attempt was made, when the 6th generation susceptibles were mated, to reduce sterility and infant deaths, while retaining susceptibility to caries, by mating rats from different strains of susceptibles in this generation. The male and female of each breeding pair were picked from different sibships, though in every case they both descended from a brother and sister in the 2nd. generation. Full brother x sister matings were used from the 7th generation to the present.

A similar procedure was followed with the resistant stock. The 5th generation resistant breeding pairs were as follows: in 4 pairs the mates were entirely unrelated to each other as far as known, 6 mates were related through one common ancestor in the 1st generation, the 2 members from 4 pairs descended from a cross between 1st generation rats, while one pair of mates were first cousins. Resistance to dental caries increased decidedly in the 6th generation, and the level of resistance was maintained in the 7th. One of the objects of these outcrosses between previously inbred resistant strains was to incorporate in one line genes for resistance that had hitherto been in different lines. This aim was apparently realized. Subsequent generations of resistant animals have been bred from full brother x sister crosses.

There are serious obstacles in the use of selection to build a desired type. The behavior of an organism (that is, its phenotype) is a very fallible index of its genetic constitution (genotype). Without enumerating the causes for this phenomenon, we will review evidence proving that it is a fact. Erwin has shown that an animal's ancestry and its own behavior are not adequate criteria for selecting breeders in this experiment, but that one must study its *offspring*, must *progeny test* it, in order to assay its genes.

A few examples from Erwin's contribution will illustrate this fact. Several females from the 3rd generation of our susceptible line were mated with susceptible male 531 from the same generation, whose caries time was 25 days. Those females producing 5 or more young are cited in Table I, together with the number of young each bore, and the average number of days required by each sibship to produce dental caries. In spite of the fact that the caries times of the mothers ranged from 25 to 86 days, the average numbers of days required by their litters to develop caries were fairly uniform, and the differences not statistically significant, except perhaps for the 5 young borne by female 337. The females seemed to have about the same genotypes, regardless of the range in their phenotypes. It seems likely that male 531 and females 344, 526, and 371 carried genes for resistance not revealed by their phenotypes. Perhaps the resistance genes introduced by the male supplemented those from these 3 females.

Table II shows the results of mating 3 third generation susceptible females with a 2nd generation resistant male. In spite of the fact that the father is the same

in all 3 matings and the range in caries time for the mothers is only 1 week, progeny averages differ in 2 instances by 25 and 35 days, and these differences are probably statistically significant.

The male was the same animal in each of the above 2 experiments, so that comparisons were possible between the different females which were mated to the same male. Thus the crosses provided progeny tests of individual females. Building the susceptible and resistant lines, however, involved selecting pairs of breeders, so that every sibship was a progeny test of its 2 parents. The principle is the same, nevertheless, in both instances. Immediate ancestry, the pheno-

TABLE I

All the females were 3rd generation susceptibles that were mated with male 531 whose caries time was 25 days.

FEMALE	CARIES TIME	NUMBER OF YOUNG	AVERAGE NUMBER OF DAYS REQUIRED FOR CARIES IN PROGENY
#470	75	17	59.35 ± 5.25
#518	72	11	70.18 ± 5.05
#499	86	6	50.00 ± 7.27
#479	58	10	67.90 ± 6.76
#471	59	13	69.15 ± 5.56
#498	56	7	60.00 ± 6.01
#337	29	5	43.60 ± 3.92
#344	31	6	57.00 ± 4.66
#526	25	11	62.00 ± 5.46
#371	25	12	66.58 ± 6.71

TABLE II

Third generation susceptible females were mated with male 305, a 2nd generation resistant male, whose caries time was 185 days.

FEMALE	CARIES TIME	NUMBER OF YOUNG	AVERAGE NUMBER OF DAYS REQUIRED FOR CARIES IN PROGENY	DIFFERENCES
#508	29	12	76.08 ±7.00	35.39 ±9.50
#536	35	17	111.47 ±6.42	25.03 ±8.35
#462	30	18	86.44 ±5.34	

types of the individuals being appraised, and the average performance of the offspring must all be considered in deciding whether a breeding pair should be included in the lines of descent of the inbred susceptible or resistant stock.

A peculiar phenomenon, for which we have no satisfactory explanation, has occurred in a few instances. In these cases one litter from a breeding pair showed a degree of resistance to caries markedly different from other litters of that pair, even though the method of handling the rats did not change as far as could be discovered. Are occasional changes in the virulence of lactobacilli in the mouths of such exceptional litters responsible for these anomalies? This seems improbable because the nasal speculum used in examining the teeth is transferred from

rat to rat without intervening sterilization. Chance combinations of genes might occasionally yield such results.

RESULTS

Let us turn now to the first objective of this research. Have these experimental procedures proved that there are inherited differences with respect to caries resistance in the albino rat?

Figs. 1 and *2* show the distributions of all individuals in the generations whose records are complete to date—12 generations for the susceptible line and 7 generations for the resistants, the first generation being the same group of animals for each. The rats were grouped into 14 day classes in preparing the data for graphing, and these 14 day classes are indicated along the abscissae in the figures.

The number of rats used in plotting each of the curves follows:

Generation	Susceptible line	Resistant line
1	116	
2	125	129
3	148	226
4	126	184
5	117	189
6	142	190
7	137	275
8	97	
9	86	
10	139	
11	74	
12	166	

It requires a much longer time for a generation of resistant rats to complete their records than for a susceptible generation, so that there are fewer generations of the former to report than of the latter. More resistants than susceptibles have been reared in each generation, because it is proving to be difficult to develop a homogeneous resistant population, while such a susceptible type has been approximated. Hence there must be a larger group of resistant animals from which to select breeders.

The caries time of the first generation ranged from the 15–28-days class to the 197–210-days class. (*fig. 1*). The mode was at 43–56-days, the mean 70 days, and the curve was decidedly asymmetrical. Though we did not at the time realize it, the first generation was relatively susceptible to caries. It would have been better had there been a larger proportion of resistant individuals at the outset, but at the time we had no basis for judging as to how resistant a resistant rat would be.

Selection in one direction for caries susceptibility and in the other for resistance produced results at once. The range of the 2nd generation susceptibles was from the 15–28 to the 155–168-days class, inclusive, with the mode at 43–56-days. The range for the 2nd generation resistants was from the 29–42-days class to the 295–308-class, followed by a long gap to 644 days, at which time male 401 died

without caries. The mode was at 85–98-days. Both curves were markedly skewed, and the resistant curve had begun to develop the long right end which in subsequent generations continued to invade the higher resistance classes.

Third generation susceptibles extended from the 15–28-days to the 85–98 class, the mode being at 29–42. The most susceptible third generation resistants came in the 29–42-days class; the upper limit was at 673-686, with 20 classes between it and class 211–224 containing no individuals at all, and 12 classes in this region having only one or two rats each. The resistant mode was the 85–98 class.

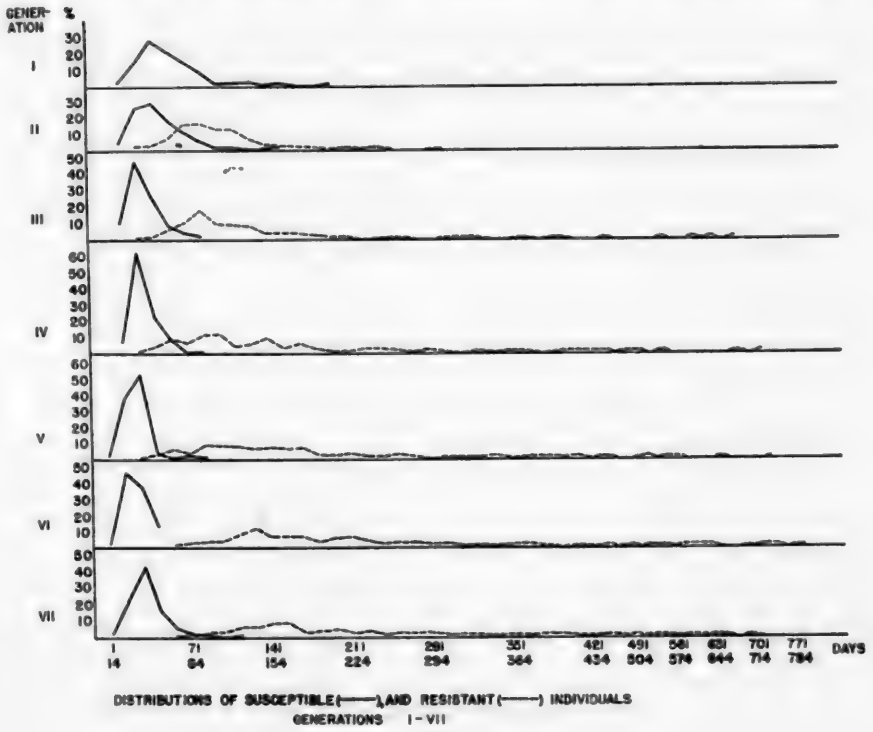
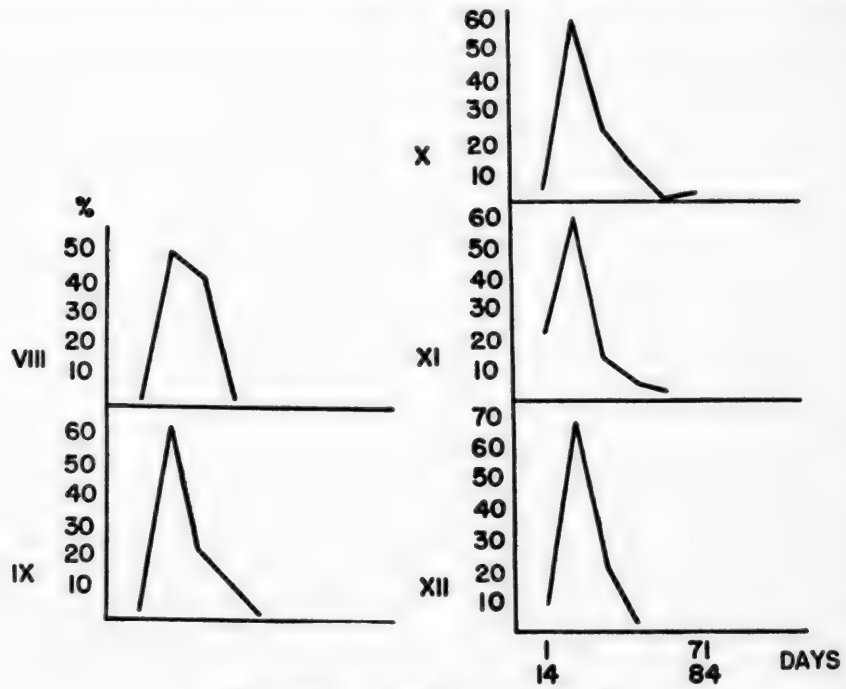


FIG. 1

The curves for the 3rd and 4th susceptible generations were very much alike. The same was true for the 3rd and 4th resistant distributions, except that the mode of the latter moved one class to the right (to 99–112), and only 14 classes between 211–224 and 673–686 were without individuals.

The 5th generation susceptible curve showed an obvious trend to increasing susceptibility. It extended from class 1–14 to class 85–98, the mode being at 29–42, though only 8.5% of the rats were more resistant than the mode. The curve for the 5th generation of resistants suggests a very slight shift toward greater resistance, though it does not differ greatly from the distribution of the 4th generation.

The 6th generation susceptibles dropped classes 57–70, 71–84, and 85–98; the mode shifted 1 class to the left, to 15–28. The resistant population of the 6th generation moved perceptibly toward greater resistance. The 29–42-days, and the 43–56-days classes disappeared, the mode changed by 3 classes to the right (to 127–140), and the upper limit of the distribution was extended to 787 days. It will be recalled that in the production of the 6th generation resistants close inbreeding was temporarily abandoned, and slightly related or unrelated 5th generation rats were crossed in the hope that genes for caries resistance from the



DISTRIBUTION OF SUSCEPTIBLE INDIVIDUALS
GENERATIONS VIII-XII

FIG. 2

different inbred strains might be incorporated in one line, causing a sudden rise in resistance, which might be fixed by further inbreeding. Such a result seems to have been achieved in the 6th generation, though it will be more evident when the means of sibship averages are discussed.

The 7th generation susceptible rats showed greater resistance to caries than did the 6th generation. This may have been due to crossbreeding the 6th generation individuals with other susceptibles in the same generation to increase reproductive efficiency. The higher resistance was temporary, however. The mode of

the 7th generation of resistants again moved to the right, and was at 155–168, though there was no other marked change from the 6th generation. The resistant curves are peculiar and interesting. Viewed successively, they remind one of an earthworm slowly extending itself and crawling to the right, toward greater resistance.

Variability remained consistently low in the 8th, 9th, 10th, 11th, and 12th susceptible generations—so low that there was little overlapping with the 7th resistant generation (*fig. 2*). This persistent low variability suggests that the susceptibles have now become homozygous for all, or nearly all, the genes responsible for very early caries. Perhaps the remaining variability is due entirely to minor fluctuations in factors other than the genes, and that this variability may prove to be of use in estimating the effects of the environment on the resistant line when the time comes for mathematical analysis of the data.

Caries is frequently "explosive" in the susceptible stock as contrasted with the resistant. For example, if a small cavity is observed at the first examination of a susceptible rat, 2 weeks later most of the crown of the tooth may have disappeared. In resistants, cavities appear late and are likely to remain relatively small. The differences in the rate of growth and number of cavities after they appear deserve more precise quantitative study than we have been able to give.

An interesting anomaly appeared in the 8th susceptible generation, which has not been plotted on *fig. 2*. Female 1989 was placed on the caries producing diet at 35 days of age, and died after having been subjected to it for 499 days. There can be no doubt about the absence of macroscopic caries, because all the last 12 fortnightly examinations failed to reveal any cavities. The average of sibship means for this generation was 29 days. This individual had been treated the same as all the other 97 animals of this generation, so it is not likely that its marked deviation was due to any environmental factor. Mutations are rare; consequently this variant may have been caused by a mutation in only 1 gene. If this is true, then 1 gene can affect a change of as much as 470 days in the caries time of a rat. This suggests that a relatively small number of gene differences may be responsible for the contrast between our susceptible and resistant lines. This theory is supported by the ease with which a rather homogeneous susceptible stock has been attained, but is opposed by our difficulties in securing a resistant strain of low variability.

There appears to be *complete immunity* to caries in some rats, which leads one to suspect that with time, resources, and skill a strain might be built which would never develop cavities when fed our caries-producing diet. Eighteen of the resistant animals (5 males and 13 females) died without developing visible tooth cavities after being subjected to the coarse rice diet for from 502 to 801 days. In our computations the "caries time" for such animals was considered as the number of days between the introduction of the cariogenic diet and the last observation of the teeth before death. We hope to create an immune strain which we can cross with the homozygous susceptibles, then estimate the number of gene pairs in which the 2 lines differ by observing the distribution in the F_2 and back-cross populations.

Size of sibship and the average resistance of sibships in the inbred resistant line are apparently negatively correlated; the more young in a sibship the sooner the teeth decay; the smaller the sibship the greater its mean resistance. This relationship is probably in part due to mating resistant animals soon after they develop cavities, so that relatively susceptible rats are mated young and produce many offspring, while the more resistant breeders are older when bred, and as a consequence of age have smaller families. This source of error is theoretically important, and may have to be considered in a later publication, but it has been of little consequence in the comparisons made here between 7th generation susceptibles and resistants.

Errors introduced by the variations in sibship size have been reduced in the data presented in Table III, where the average of mean sibship caries times is given for the susceptibles and the resistants in each generation. For example,

TABLE III

Averages of sibship means. (The sibships used comprise 5 or more rats each)

Average of the first generation, 70 days

GENERATIONS	SUSCEPTIBLES		RESISTANTS		DIFFERENCES BETWEEN AVERAGES
	Averages of means	Numbers of sibships used	Averages of means	Numbers of sibships used	
2	57 days	8	116 days	8	59 days
3	43 "	11	142 "	18	99 "
4	37 "	13	168 "	16	131 "
5	32 "	14	186 "	11	154 "
6	29 "	11	248 "	14	219 "
7	38 "	11	245 "	22	207 "
8	29 "	9			
9	30 "	9			
10	30 "	9			
11	22 "	6			
12	24 "	15			

there were 11 sibships in the 7th generation of the susceptibles. The mean time required for the animals to develop caries was computed for each sibship, the 11 means were averaged, and this average, 38 days, was entered in the table. Sibships with less than 5 rats were excluded. In this way an average time required to develop tooth cavities was secured for each generation of susceptibles and resistants, with the effect of size of sibship eliminated, except in so far as this factor might have affected the mean of a sibship in some indirect manner. The average for the first generation was 70 days. The susceptible averages declined with but slight fluctuations from 57 days in the 2nd generation to 24 days in the 12th. The averages have varied little from the 4th generation on. It is obvious that the lower limit of susceptibility has been approximated. The resistant averages steadily increased from 116 days in the 2nd generation to more than twice this length of time in the 6th and 7th. The differences between susceptible and resistant averages rose from about 2 months at the 2nd generation to approximately 7 months in the 6th and 7th generations.

The sibship means for both the susceptible and the resistant lines are plotted in *fig. 3*. Each symbol on the graphs represents the mean of 1 sibship. The averages of succeeding generations are connected by lines. The steady divergence of these lines, as well as the increasing values in the right-hand column of Table III, show unmistakably that our system of mating has, up to the present, produced a growing difference between susceptibles and resistants. It will be

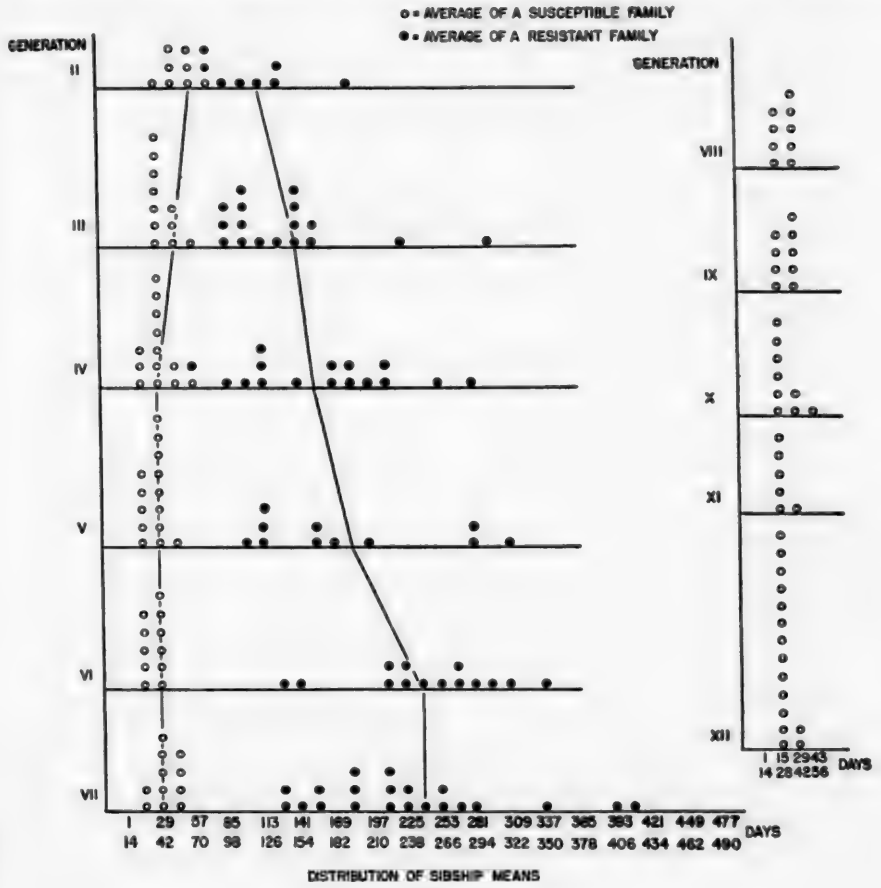


FIG. 3

noted that in the 5th, 6th, and 7th generations there was no overlapping between the sibship means of these 2 lines.

The experienced statistician will readily concede that by the 7th generation the difference between the susceptibles and resistants had undoubtedly become statistically significant; that is, it could not be regarded as a consequence of chance. Experience in observing many hundreds of these rats gave us also this impression. However, we have mathematically tested the reliability of the difference between the average of 38 days for the 7th generation susceptibles,

and the 245 day average of the 7th generation resistants. The t value for this difference of 207 days is 7.31, which signifies that the odds against its being a matter of chance are greater than 2,000,000 to 1.

We likewise computed t for the difference between the 2 sibship averages in the 7th generation when these averages were obtained from weighted sibship means. Each sibship mean was multiplied by the number of rats in that sibship, the products were added, and the sum divided by the total number of rats involved. The 2 averages were 37 days and 242 days. These are substantially the same as the values secured by averaging unweighted sibship means, showing that at least in the 7th generation, size of sibship was not a distributing factor of consequence. This might not be the case in other generations. The t value for the difference

TABLE IV

Data on F_1 and F_2 progeny from P_1 cross resistant male x susceptible females

P_1 male 305 (caries time 185 days) was used

NO. AND CARIES TIME OF P_1 FEMALES	NUMBER OF F_1 PROGENY	MEAN CARIES TIME FOR F_1 PROGENY	NUMBER OF F_2 PROGENY	MEAN CARIES TIME FOR F_2 PROGENY	COEFFICIENT OF VARIATION	
					F_1's	F_2's
500 (30 days)	23*	108.09 ±14.26*			61.89*	
	22	94.59 ± 4.83	73	76.23 ±4.36	23.40	48.12
508 (29 days)	12	76.08 ± 7.00	7	58.86 ±8.90	33.58	37.54
537 (35 days)	18	95.28 ± 8.41	149	85.83 ±3.12	36.36	44.20
536 (35 days)	17	111.47 ± 6.42	85	102.36 ±6.21	23.03	55.67
462 (36 days)	18	86.44 ± 5.34	78	81.31 ±5.91	26.62	62.65
Totals and means	88*	97.33 ± 4.65*			44.59*	
	87	93.79 ± 3.06	392	86.29 ±2.34	30.24	53.70

* These data include F_1 female 89 (405 days). Unstarred data do not include her.

of 205 days is 37.5, indicating that this difference is highly significant. Available tables do not deal with such enormous odds against a chance event.

Thus when judged by any criterion, genuine hereditary difference between the latest comparable generations of resistants and susceptibles has been proved.

GENETIC MECHANISM

The second objective of this research is to determine, if possible, the number of gene pairs responsible for this difference, and the manner in which each gene acts. This may prove to be a very difficult task. Erwin bred F_1, F_2, and back-cross generations, but the P_1 breeders he selected from the early generations of the susceptibles and resistants were probably not homozygous for the genes concerned with caries, so his results were not conclusive.

Table IV presents Erwin's data secured from mating a 2nd generation resistant

male (♂305) with five 3rd generation susceptible females. Male 305 developed caries at 185 days after starting to eat the coarse rice mixture, while the caries times for the 5 females ranged from 29 to 36 days. The F_1 population averaged 97.33 or 93.79 days, depending on whether or not one includes one very exceptional female whose caries time was 405 days. The F_2 progeny were produced from crosses in which all of the F_1 females from a given P_1 cross were mated with one of their male sibs. In each case the F_1 male selected most nearly approximated the average caries time of the first litter of the sibship. There were 392 F_2 rats, with an average caries time of 86.29 days. At the time the report was written, 18 F_2 animals had not developed cavities, though all had been on the experimental diet over 100 days, 5 of them over 200 days. Thus the average given for the F_2 population is too low. The coefficient of variation for the F_1 group was 44.59 or 30.24, the difference being due to the inclusion or exclusion of the exceptional animal. In the F_2, the coefficient of variation rose to 53.70.

A comparison between the means and coefficients of variation of F_1 and F_2 generations provides important evidence as to whether a number of gene pairs, having similar effects, is involved in determining a trait. If the F_1 and F_2 means are approximately the same, but the coefficient of variation of the F_2 population significantly exceeds that of the F_1's, then there is evidence that a number of similar gene pairs are at work. As far as they go, these data support such a multiple gene theory.

A back-cross population of 45 rats was secured by mating 4 F_1 males (from the the P_1 crosses resistant male x susceptible females) with four 5th generation susceptible females. The back-cross mean was 63.31 days, which is 34 days less han the F_1 mean. Thus this back-cross generation regressed somewhat toward the susceptible line's level. In general the distributions in the F_1, F_2, and back-cross populations fit the hypothesis of multiple similar gene pairs, though the probability that Erwin did not start with homozygous P_1 animals makes it impossible to use his data for estimating the number of pairs involved.

We are now trying to secure either a homozygous resistant line (as identified by low variability within this line) or a caries immune line, to cross with our susceptibles, so that F_2 and back-cross generations adequate for computing the number of gene pairs can be obtained. There may be only 2 or 3 gene pairs, or there may be many. The evidence at hand is conflicting. A homogeneous susceptible line was secured with surprising ease. This favors the theory that but few genes are required for susceptibility, for it would be much easier to get homozygosity for 2 or 3 pairs of genes than for a larger number. The mutation (female 1989) in the 8th generation of susceptibles points in the same direction. Mutations in genes are rare, thus such a marked variation would probably be due to alteration in only 1 gene. If the resistance of female 1989 was due to a change in a single gene, then the differences between the susceptible and resistant stocks might well be caused by a very small number of such genes.

On the other hand, the persistent variability of the resistant line, and the slow creep of the curve toward greater resistance arouse the suspicion that many gene pairs may be concerned, and that the ineffectiveness of selection in securing homo-

geneity in resistance may be due to the difficulty of getting rats having all of the many genes for resistance. It should be recalled that we started with a markedly susceptible 1st generation, and that the ease in building the homogeneous susceptible line may be attributable to the presence of many favorable genes at the outset. Only further study can solve this problem.

DISCUSSION

Another question is, "What are the anatomical and physiological differences between the susceptible and resistant types?" Genes produce their effects by initiating events which lead to the development of structures, and to chemical processes. Both the susceptibles and the resistants have consumed the same kind of food, drunk water from the same source, lived in the same kind of cage, and been handled by the same caretaker in the same building. Are there structural differences between the teeth of the two types? This matter should be investigated. Are the bacterial flora of the mouths the same? Dr. Philip Jay of the University of Michigan is studying this very important question. Perhaps the chemical constitution of the saliva differs in the two types. A competent physiologist might well use our rats for the necessary analyses.

For the first time, no doubt, in the history of dental research there is a uniform strain of rats known to be highly susceptible to dental caries when fed a specific diet. This strain should be used to test the effectiveness of various chemical means for preventing caries. Experiments are being conducted here with topical applications of fluoride solutions to find out whether caries can be delayed when the susceptibles are fed the coarse rice mixture. If a sufficient number of substances is tested, perhaps some very effective, cheap, and harmless compound can be found, much better than anything now known, for preventing tooth decay.

This investigation has provided a good example of the determination of a trait both by the genes and by the environment. The appropriate genes delay, or even prevent, dental caries. Grind the rice to flour fineness, and a similar result is usually obtained even with highly susceptible rats.

Do our results prove anything concerning the role of heredity in human caries? The answer is obviously, "No". The rat is a rodent and man is a primate; they are only distantly related phylogenetically. Nevertheless, the pathologist experiments with guinea-pigs, rabbits, and dogs so that he may formulate hypotheses as to what may be true for human beings, and these hypotheses often prove to be correct. At least our results lend strong support to the idea that there may be hereditary differences in man with respect to dental caries. Such a hypothesis must be tested by critical observations on human beings before any conclusions are drawn. If it should be found that some human families show early and high hereditary incidence of dental caries, their members should visit the dentist regularly so that prompt repair of cavities may preserve their natural teeth longer.

SUMMARY

1. The conclusion of Hoppert, Webber, and Canniff that a diet of coarsely ground rice, powdered whole milk, alfalfa leaf meal, and sodium chloride will induce dental caries in rat molar teeth, has been repeatedly confirmed.

2. If the rice component of this diet is ground to flour fineness, caries is at least delayed.

3. The time required to develop tooth cavities is not a reliable index of the genotype of the individual rat.

4. A caries susceptible strain and a caries resistant strain of rats have been produced by selection, progeny testing, and close inbreeding. Heredity is therefore an important factor in the development of rat caries.

5. There are reasons for believing that multiple factors are responsible for differences in caries resistance, but further experimental work is required to test this hypothesis, to identify the number of gene pairs, and to discover the physiological and anatomical effects of these genes.

6. A highly susceptible type of rat has been secured which is excellent material for studies on the chemical prevention of tooth decay.

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